

**Society for Pediatric Pathology
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Abstracts are listed in presentation order, beginning with Platform Presentations.

Platform Presentations

1 Complications Following Stages I And II Hybrid Repair For Hypoplastic Left Heart Syndrome (HLHS)

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Background: Staged hybrid procedures represent a recent advancement in repair of hypoplastic left heart syndrome (HLHS). Complications and cause of death following stages I and II have not been well-documented at autopsy.

Design: Autopsy reports were reviewed for 12 consecutive patients (November 2002 through April 2007) who underwent repair for HLHS (4 males and 5 females expired following stage I and 3 males following stage II). Post-procedural complications and causes of death were recorded.

Results: The mean age at death was 57 (stage I, range 0-180) and 147 (stage II, range 77-210) days. The mean interval between procedure and death was 44 (stage I) and 13 (stage II) days. Autopsies had no restrictions (n=5 stage I, n=3 stage II), thorax only (n=3) or chest and abdomen only (n=1). Complications following stage I and not resulting in death included large vessel thrombosis (n=2), recent cerebral ischemic injury (n=2), recent myocardial ischemic necrosis (n=2) and diffuse alveolar damage (n=1). Remote ischemic cerebral injury in 1 patient, who expired at age 10 days, suggested inadequate oxygen supply in-utero. Death was due to cardiac arrhythmia with myocardial fibrosis (n=2) or myocyte disarray (n=1) likely accounting for the arrhythmia, pneumonia (n=2), bowel necrosis (n=1), pulmonary thromboemboli (n=1) or hemopericardium due to left atrial appendage tear (n=1). One patient, who died of pneumonia, had recent myocardial necrosis as a condition which contributed to death. One patient died following onset of arrhythmia with no identified myocardial substrate. Complications following stage II and not resulting in death included recent cerebral ischemic injury (n=1), large vessel thrombosis (n=3) and pneumonia (n=1). Death was due to ischemic myocardial injury (n=2) or pulmonary hemorrhage (n=1).

Conclusion: The cardiovascular system was a frequent site for complications and cause of death following stages I and II hybrid repair for HLHS. Large vessel thrombosis was present in all patients following stage II. In patients with fatal arrhythmia following stage I, autopsy frequently documented a myocardial substrate which likely accounted for the arrhythmia.

2 Respiratory Syncytial Virus (RSV) Pneumonitis in Mice is Associated with Persistent Inflammation, Chronic Respiratory Dysfunction, and RSV RNA Persistence

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Background: RSV is the leading cause of respiratory infection in infants and children worldwide. There is a strong association between RSV infection in infancy and the subsequent development of recurrent wheezing and recurrent airway hyperresponsiveness. To shed light on the pathogenesis of RSV infection and the associated airway hyperresponsiveness, we studied the lung histology and respiratory function in mice with RSV pneumonitis.

Design: Mice were infected intranasally with RSV. Controls were inoculated with medium. Whole body plethysmography was used to monitor the respiratory function. Groups of mice were sacrificed at days 0, 1, 4, 5, 11, 14, 21, 43 and 70 after infection and whole mount sections of the lungs were stained with H&E, Trichrome, PAS, and immunohistochemical stained for RSV. The persistence of RSV RNA in the lungs was tested by PCR.

Results: The earliest histologic changes (day 1 postinfection) were observed in the pulmonary vessels with enlargement of endothelial cells, binding of leukocytes to the endothelium, perivascular edema, and a scant neutrophilic infiltrate in the perivascular space. A slight intra-alveolar infiltrate composed of macrophages and neutrophils was also observed. These changes were more marked at day 4 postinfection and, in addition, a progressive infiltrate composed predominantly of lymphocytes with some neutrophils was also observed involving the space around small and larger airways and vessels. The inflammation peaked between days 4 and 5 postinfection. By day 14, there was a lymphoplasmacytic infiltrate limited to the larger airways and vessels, and only a few macrophages in the alveolar spaces. Mucus hyperproduction was demonstrated at days 5 and 14 postinfection. From day 21 on, the inflammation was composed of mononuclear cells containing abundant plasma cells, and involved only the large vessels and airways. No inflammation of the alveolar spaces was observed beyond day 21 postinfection. A persistent scant inflammatory infiltrate, composed of mature lymphocytes and plasma cells, was identified around larger airways and vessels as late as day 70 postinfection. By whole body plethysmography, infected mice also demonstrated persistent respiratory dysfunction for as long as day 70 postinfection. Further, RSV RNA persistence in the lungs was demonstrated by PCR beyond day 70 postinfection. Immunohistochemical staining demonstrated that early in RSV infection in the mice (day 5), viral protein can be detected in the alveolar lining epithelium and alveolar macrophages.

Conclusion: RSV infection in the mouse is associated with persistent inflammation, mucus hyperproduction, persistent airway dysfunction, and persistent RSV RNA in the lungs.

3 Congenital cystic adenomatoid malformations demonstrate selective epithelial proliferation during fetal life

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Background: The etiology of congenital cystic adenomatoid malformations (CCAMs) is unknown. Rare resections in fetal life provide a unique opportunity to study their development. Prior studies have demonstrated a general increase in proliferation but were limited by a paucity of fetal specimens. We hypothesized that a selective increase in epithelial proliferation during fetal development might play a role in CCAM pathogenesis.

Design: Tissue microarrays (TMAs) of CCAMs resected during fetal (n=15) and postnatal (n=13) life using formalin-fixed paraffin-embedded tissues were created (triplicate sample punches). Control lung tissues from adjacent non-lesional areas and age-matched autopsy tissues (n=20) were included on each TMA. TMAs were double-labeled for cytokeratin AE1/3 and Ki67 with adequate controls. The proliferative index for airspace epithelium was calculated as: # Ki67(+)/AE1/3(+) epithelial cells/total # AE1/3(+) epithelial cells (10 airspaces). The proliferative index for mesenchyme was calculated as: # Ki67(+)/AE1/3(-) interstitial cells/total # interstitial cells (400 cells per core). Statistical analysis was performed using Prism 4.0 (GraphPad software).

Results: Epithelial proliferation was increased in fetal CCAM tissue compared to non-lesional (1.6 fold increase, P<0.0001, ANCOVA test) and autopsy (2.7 fold increase, P<0.0001, ANCOVA test) tissues. Non-lesional epithelium showed an intermediate increase in proliferation versus autopsy controls (1.8 fold increase, P<0.0001, ANCOVA test). In contrast, mesenchymal proliferation was identical in fetal CCAM and non-lesional tissues, although both had increased proliferation over autopsy controls (1.6 fold increase, P<0.0001, ANCOVA test). The rate of decline in proliferation in both fetal epithelium (-0.056 units/week) and fetal mesenchyme (-0.028 units/week) was similar in all groups. Postnatal CCAMs showed no increase in epithelial or mesenchymal proliferation versus controls.

Conclusion: Fetal CCAMs demonstrate a selective increase in epithelial proliferation during gestation. In contrast, mesenchymal proliferation shows no difference between CCAM and non-lesional tissues. Thus, we speculate that mesenchymal proliferation is reactive within fetal lungs harboring CCAMs, whereas increased epithelial proliferation during development, either as a primary process or in response to external factors, plays a central role in CCAM pathogenesis.

4 Multicystic Renal Dysplasia: With Or Without Obstruction? That Is The Question

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Background: Multicystic Renal Dysplasia (MCRD) features abnormal nephron differentiation and renal development. The best characterized type is that related to urinary tract obstruction (UTO). However, cases of MCRD, such as those in

Meckel-Gruber (MGS), Ivemark (IVS) or Joubert syndromes, or in metabolic disorders like glutaric aciduria-II (GA-II), Zellweger synd. and CPT2 deficiency are not associated with UTO. The pathobiological differences among MCRD cases of different etiology have not been highlighted; their classification is confusing, and their pathogenesis is poorly understood. Aberrant apoptosis and cell proliferation have been observed in cases with UTO, suggesting that obstruction may alter proper molecular renal development, but our mechanistic understanding of MCRD is incomplete.

Design: 23 cases of MCRD (14-37 wks of gestation; mean-23.3 wks) were studied; 13 were associated with UTO (Group 1). In 10, UTO was ruled out (Group 2) with the following diagnoses: GA- II (5), MGS (2) and IVS (1). Kidneys from 13 age-matched controls were included. Histologic sections were evaluated for degree of architectural distortion, amount of large cysts and of normal parenchyma. Detection of Ki-67 and Bcl-2 was performed in cases and controls by IHC. Cytokeratins 5/6, 7 and 8 were studied in an attempt to determine the nephron segments from which the cysts originate.

Results: The 2 groups featured extensive architectural disorganization, cysts and immature mesenchyme. However, severity was greater in Group 1. In most cases, with and without UTO, Ki-67 detection was increased in the cystic epithelium, compared to the normal tubule epithelium in controls. Bcl-2 expression was increased in both groups. CK 7 was strongly expressed also in both groups, supporting a distal nephron origin for most cysts.

Conclusion: There are significant differences in severity between MCRD with and without UTO. However, even without UTO, the disorganization of renal development takes place altering epithelial cell proliferation and survival, suggesting that pathogenetic mechanisms are shared between the two groups. Other mechanisms for cyst formation and mesenchymal disorganization in this phenotype remain unknown. The lack of UTO in Group 2 reveals that we ignore part of the pathogenetic equation, which deserves further research. We propose that MCRD should be divided into two groups: obstructive MCRD (OMCRD) and non-obstructive MCRD (NOMCRD).

5 Spectrum of lung disease in immunocompromised children (2-18 years): A multi-institutional study of the Children's Interstitial Lung Disease (ChILD) Research Cooperative

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Background: The ChILD Research Cooperative has used a classification scheme for diffuse lung disease in young children (<2 years of age) to describe the spectrum of disease, clinical presentation and outcome in affected children. Using the same multi-institutional collaborative approach, we applied a similar scheme to biopsies from older children (2-18 years); results of immunocompetent children in this group have been previously reported. We now report results for immunocompromised children.

Design: All lung biopsies performed for diagnosis of diffuse

lung disease in children 2-18 years in a 4 year period were reviewed and categorized. Clinical data was obtained by chart review. Immune compromise was defined as congenital or acquired immune deficiency, chemotherapy for malignancy, or post-transplant status.

Results: 95 biopsies from 91 immunocompromised children (48.2% of total biopsies) were reviewed. Conditions leading to immunocompromise were bone marrow transplantation (37), chemotherapy for malignancy (26), organ transplantation (12), and immunodeficiency syndromes (14) including chronic granulomatous disease (CGD) (6), common variable immunodeficiency (5), and 3 others. 8/95 biopsies were unclassifiable, two had diagnoses unrelated to immunocompromise; one post renal transplant with lung growth abnormality, and one post liver transplant with septic emboli. Infection was the commonest diagnostic category (39) with fungus the most frequently identified agent (15); 17 had changes related to therapeutic intervention; 8 had changes of transplantation/rejection syndromes; 12 had active changes of undetermined etiology; 9 had lymphoid infiltrates related to immunodeficiency. Two with immunodeficiency syndromes also had clinical diagnoses of collagen vascular disease. There was no patient with HIV in this cohort.

Conclusion: Lung biopsy from immunocompromised children (2-18) accounts for almost half the biopsies for diffuse lung disease in this cohort, whereas in infants it accounted for 15%. The commonest condition associated with immunocompromise in older children is bone marrow transplantation (40.7%). Infection was the commonest diagnostic category (45.9%), twice as frequent as changes related to therapeutic intervention (20%).

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6 Neuroendocrine Cell Hyperplasia of Infancy (NEHI): Defining the Histologic Spectrum

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Background: Neuroendocrine cell hyperplasia of infancy (NEHI) is an idiopathic lung disorder distinct to infants and young children, and classically presents with persistent tachypnea, crackles and hypoxia and segmental ground-glass opacities by high-resolution CT (HRCT). Lung biopsy findings are typically minor and nonspecific; pathologic diagnosis rests on demonstrating the presence of increased neuroendocrine cells within bronchioles without other pathologic processes present (Deterding et al. 2005). Increasing clinical suspicion for NEHI and experience in pediatric thoracoscopy has resulted in lung biopsies with a wider range of histologic findings than that previously described. Our objective was to examine the spectrum of pathology in patients with NEHI.

Design: Lung biopsies were reviewed from 11 patients with the characteristic clinical presentation and distinctive imaging appearance of NEHI. Quantification of bombesin immunopositive cells was performed as previously described.

Results: The mean age at biopsy was 15.1 months (4.8-43 months). Four of 11 cases had only nonspecific changes typical of NEHI including increased alveolar macrophages, mild increase in airway smooth muscle and mild periairway lymphocytic aggregates. The remaining 7 cases had additional

findings including patchy lymphocytic bronchiolar inflammation and mild periairway and subepithelial fibrosis. One case had prominent lymphoid hyperplasia with reactive germinal centers. Three of the 7 cases with airway injury had confirmed viral infection prior to biopsy (2 RSV, 1 parainfluenza) and 2 others were clinically suspected; in contrast none of the classic NEHI cases had clinical evidence of a viral illness. All 11 cases had increased bombesin immunopositive cells in terminal airways and prominent NEBs in the lobular parenchyma. Quantification of bombesin staining revealed an average of 9.5% immunopositive cells in airway epithelium within classic NEHI cases. In biopsies with airway injury the average number was 11.6% in noninjured airways versus 1.6% within airways with active inflammation. In 3 cases biopsies were obtained from radiographically distinct areas of ground-glass opacities and hyperinflation; the proportion of bombesin immunopositive cells did not differ in these regions. No mortality occurred with a mean age at follow-up of 7.9 years (7 months-16 years), although 8 children remain on supplemental oxygen.

Conclusion: Presence of airway injury at lung biopsy does not preclude the clinical-radiographic-pathologic diagnosis of NEHI. Further studies are needed to determine whether the histologic spectrum reflect heterogeneity of pathogenesis or prognosis

7 Increased Number of Migratory Trophoblastic Cells in Placental Membranes with Microscopic Chorionic Pseudocysts

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Background: Placental membrane microscopic chorionic pseudocysts (MCP) are associated with clinical conditions, such as preclampsia and diabetes mellitus, and placental features, such as infarctions, laminar necrosis and global hypoxic placental injury patterns, of in-utero hypoxia. This retrospective analysis was designed to assess the amount of placental membrane migratory trophoblasts (MT), its maturity, proliferation and apoptosis in the placental membranes with MCP.

Design: Placental membrane rolls of 50 consecutive placentas with MCP (study group, SG) were compared with membrane rolls without MCP in membranes or in placental parenchyma (chorionic disc, cell island, septal and maternal floor chorionic pseudocysts) from 50 pregnancies matched for gestational age (control group, GG). The average MT cell layer thickness was determined by counting the MT cell number across the trophoblastic layer of placental membrane away from MCP in five representative sites of the membranes double immunostained for E-cadherin (which highlights the MT cell membranes but not the decidual cell membranes) and Ki-67 (proliferation marker). The rolls were also immunostained for human placental lactogen (hPL) (an intermediate trophoblastic maturation marker) and M30 (a marker of irreversible apoptosis in epithelial cells). Cells positive for Ki-67, hPL and M30 were counted in 5 representative high power fields (hpf, objective x40). Statistical differences between SG and CG were determined with ANOVA, single factor.

Results: Average gestational age in SG and CG was 35.3 weeks

in both SG and CG. The membrane trophoblastic layer in SG and CG was on average 7.1"2.8 and 4.5"1.5 cell thick, respectively ($p < 0.001$). These cell counts correlated weakly positively with gestational age in SG ($R = +0.3$), but not in CG or for all other immunohistochemistry markers in SG or CG. When corrected for the MT cell layer thickness, the Ki-67 (9.8"6.0 and 10.7"7.7), hPL (10.0"16.7 and 9.2"19.1), and M30 (3.8"4.1 and 4.7"5.4) indices (sum of positive cells per 5 hpf) did not differ statistically significantly between the SG and CG ($p > 0.05$), respectively.

Conclusion: MCP are associated with increased thickness of MT cells in the membrane trophoblastic layer, which parallels the increased number of the implantation site extravillous trophoblasts observed by others in preeclampsia. However, no differences in proliferation, apoptosis or trophoblast maturity were found between SG and CG based on the immunostains applied. Therefore, other factors, e.g. an increased avascular watershed-area of membranes, more vulnerable to hypoxia, may be implicated in pathogenesis of MCP which are more prevalent in clinical conditions at risk for hypoxia.

8 Post Mortem Imaging of Fetal Central Nervous System Malformations

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Background: Post mortem MRI of structural brain abnormalities can be used as an adjunct in the diagnosis and investigation of primary brain abnormalities, with documented advantages in the ability to detect and define image abnormalities in situ, which can be difficult to evaluate in the dissected specimen. Post mortem autolysis and the intrinsic delicacy of hydrocephalic brains or cystic malformations can further limit the capacity for detailed histopathological correlation. In this study we undertake systematic post mortem imaging of fetal brain abnormalities identified antemortem in order to identify the principle utility of such examinations, to define further its limitations, and to explore means of improving MRI-histology correlation.

Design: We imaged a series of 21 structurally abnormal fetal brains (gestational age 16-32 weeks) following post mortem extraction and fixation. Brains were imaged using a 1.5 T magnet and T1 weighted and diffusion tensor weighted imaging. Images obtained were compared to gross and histological sections. 3 structurally normal fetal brains were imaged as controls.

Results: Structural MRI identified and corroborated the gross anatomical abnormalities in all cases examined, and in addition provided a guide to neuropathological sectioning in a case of persistent cervical neurenteric canal, and in evaluation of encephalocoele content and extent. Detailed architectural correlation between histology and imaging was made possible on severely hydrocephalic and autolysed brains by sectioning the brains grossly, stabilizing the gross sections with agar and sectioning on a sledge microtome. Post mortem MRI could detect areas of polymicrogyria or cavitation affected by infarction or with abnormal cortical maturation due to adjacent cyst, but could not resolve the anatomical basis of the injury.

Conclusion: As previous work has demonstrated, post mortem MRI yields superb resolution of gross anatomical organization and can guide dissection, although histopathology remains critical for definitive examination. Precise correlation of MRI with

histology on fragile and autolysed specimens can be remarkably improved by agar stabilization and whole mount preparation.

9 Placental Pathology Correlates with Low First Trimester Serum Pregnancy Associated Placental Protein A (PAPP-A)

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Background: Pregnancy-associated plasma protein-A (PAPP-A) is routinely measured at 11-13 weeks gestation to screen for trisomy 21. Low PAPP-A is associated with adverse pregnancy complications attributable to placental disease, namely IUGR, pre-eclampsia, preterm delivery and stillbirth.

Design: This is an ongoing prospective cohort study of women with singleton structurally-karyotypically normal pregnancies with PAPP-A ≤ 0.3 multiples of median [MoM]). Each woman has a second trimester alpha-fetoprotein test, followed by second trimester ultrasound imaging of the placenta and the data are related to clinical outcomes and placental pathology.

Results: To date 66 women have been evaluated, of which 33 have delivered and 28 have had their placentas evaluated for pathologic examination. 4/28 of these women had preeclampsia. 10 of 26 (38%) who underwent second trimester ultrasound had placental parenchymal abnormalities detected. There were 7/28 (25%) live term deliveries. 9/28 (32%) infants were stillborn and there was one neonatal death. 10/28 (36%) of the babies had intrauterine growth restriction. The placental pathology findings were as follows: Only 2/28 placentas were without significant pathology. 18/28 (64%) weighed less than the 10th percentile for gestational age and 1 weighed more than the 90th percentile. 7 (25%) cords were marginally inserted, suggesting chorion regression in early pregnancy, and of these 5 were velamentous. 7/19 cords were overcoiled and 12 showed normal coiling. 9 were too short for assessment of coiling. 10/28 (36%) placentas showed infarction, and 12 (43%) showed evidence of maternal vascular insufficiency (either infarction, advanced villous maturity for gestational age or decidual vasculopathy). 6 showed distal villous hypoplasia and 6 showed intervillous thrombi. 2 showed subchorionic hemorrhage, one of which was a Breus= mole. 2 showed retroplacental hemorrhage.

Conclusions: Low PAPP-A is associated with a striking rate of developmental and vascular pathology that correlates with placental ultrasound and adverse clinical outcomes.

10 Soluble VEGF Receptor-1 Is Overexpressed In Placentas With Abruption

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Background: Placental abruption is a major risk factor for both stillbirth and preterm delivery and its etiology remains poorly understood. Circulating angiogenic factors such as soluble VEGF receptor-1 (sVEGFR1; also called sFlt1) have been shown to play a role in the pathogenesis of preeclampsia. Since the risk of abruption is increased in preeclampsia, we asked whether the expression of sVEGFR1 is increased in abruption, with or without a clinical diagnosis of preeclampsia.

Design: All placentas had a gestational age between 20 weeks and term and were submitted for evaluation within the last 3 years (2005-2007) at Brigham and Women's Hospital. A series of 31 cases with a clinical impression of abruption, confirmed by the presence of a retroplacental hematoma, were selected. Of these 31, 6 had a diagnosis of preeclampsia. In addition, 40 gestational age-matched placentas with a diagnosis of preeclampsia, without abruption, as well as 44 cases without either abruption or preeclampsia, were selected as positive and negative controls, respectively. A representative section of placental parenchyma was stained with an antibody to sVEGFR1 (Abcam, 1:400 dilution), and the staining was scored as either none, focal (5-25%) or diffuse staining (>25%), based on percent syncytiotrophoblastic staining.

Results: Of the 40 preeclamptic placentas, 36 (90%) showed at least focal staining for sVEGFR1. A significantly higher percentage of placentas with abruption (14/31 or 45%) showed sVEGFR1 staining, compared to 9/44 (20%) negative controls (P=0.04). Of the six cases of placentas with both abruption and preeclampsia, 5 (83%) showed sVEGFR1 staining (P=0.006). In addition, 9/25 (36%) of the placentas with abruption, but without preeclampsia, showed sVEGFR1 staining. However, this difference did not reach statistical significance when compared to the negative control group (P=0.42).

Conclusions: The data raises the possibility that sVEGFR1 may play a role in placental abruption. Further studies are needed to explore the relationship between preeclampsia and abruption and the potential role for angiogenic factors.

11 Adverse Obstetric And Neonatal Outcomes And Umbilical Cord Abnormalities In Placentas With Fetal Thrombotic Vasculopathy

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Background: Fetal thrombotic vasculopathy (FTV) is a placental lesion characterized by regionally distributed avascular villi, and is often accompanied by upstream thrombosis in the placental fetal vascular tree. Previous studies have shown associations of this lesion with placental insufficiency, adverse neurologic outcomes in the newborn, as well as potentially obstructive lesions of the umbilical cord. We set out to study the prevalence of specific obstetric complications, neonatal disease, and umbilical cord abnormalities in cases with FTV.

Design: 154 cases of placentas with FTV were identified in the pathology database at Brigham and Women's Hospital over the last 15 years. 111 consecutive placentas without the diagnosis of FTV were selected as controls. Both the pathology report as well as electronic medical records were used to extract the following information on all cases: gestational age, method of delivery, fetal/neonatal outcome (livebirth vs. stillbirth or neonatal death), potentially obstructive lesions of the cord (ex. True knot, nuchal cord), obstetric complications (pregnancy-induced hypertension or PIH/preeclampsia, gestational diabetes, oligohydramnios, chorioamnionitis), and fetal abnormalities (non-reassuring fetal heart tracing or NRFHT, intrauterine growth restriction or IUGR, congenital abnormalities). Data analysis was performed using Microsoft Excel.

Results: Placentas with FTV were associated with an almost 10-fold increase in rate of stillbirth/neonatal death (17.5% cases

vs. 1.8% controls; P=0.0001), a 16-fold increase in IUGR (28.6% cases vs. 1.8% controls; P=0.0001), and a 4-fold increase in PIH/preeclampsia (16.9% cases vs. 4.5% controls; P=0.0037). While the rate of potentially obstructive cord lesions was similar in both groups (20% cases vs. 18% controls), there was a 7-fold increase in the presence of oligohydramnios in FTV placentas (13% vs 1.8%). Most interestingly, FTV was associated with a 6-fold increase in congenital heart abnormalities (16.9% vs. 2.7%). There was no significant difference in the rate of C-section, NRFHT, chorioamnionitis, or gestational diabetes between the two groups.

Conclusions: FTV is associated with a significantly higher rate of adverse obstetric and neonatal outcomes. This study points to abnormal fetal circulation, either in the form of congenital heart disease or oligohydramnios predisposing to cord compression, as a risk factor for FTV.

12 Performance characteristics of criteria for fetal vascular thrombosis in stillbirth placentas

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Background: Recently proposed criteria identify fatal restriction of umbilical blood flow in stillbirth placentas. This study tests performance characteristics of four histologic criteria identified in discriminating stillbirth placentas due to fetal vascular thrombosis from placentas with postmortem vascular involuntional changes.

Design: 25 placentas were randomly selected from two previous series that identified histologic criteria for fetal vascular thrombosis (FVT) as a cause of stillbirth, then tested criteria reproducibility in an independent stillbirth placenta set. Control placentas included other known causes of demise, and stillbirths of unknown cause. Causes of demise (FVT, other, unknown) were previously categorized by consensus of two senior authors TB, MP. In this study, one senior author TB) and four Pediatric Pathology fellows evaluated placentas for the following characteristics: chorionic plate (CP)/stem villous (SV) vascular ectasia, CP/SV thrombosis, villous-stromal karyorrhexis, and avascular villi. Each histologic finding, if present, was scored by regional or global distribution. Cases were scored as: changes of FVT, postmortem demise but not FVT, or unknown. P values for each characteristic were calculated against previously determined cause of demise, and kappa statistics were calculated for interobserver variability. For calculating p values, a characteristic was considered present if 4 or 5 observers scored it. For kappa statistics, the senior author's observations were scored against the fellows' observations for each characteristic. The latter was scored as present if 3 or all 4 fellows concurred. The senior author's intraobserver variability for each characteristic was also calculated.

Results: 15 placentas were from previously diagnosed FVT; 10 placentas were from IUD of other known, or unknown but not FVT, cause.

	CP/SV ectasia	CP/SV thromb	vil/str karyo	avasc vil	Imp v. Dx FVT
P value	.0361	.0025	#s too small	#s too small	.0005
kappa	.52	.8	.73	.8	.84

Conclusions: P values were highly significant in predicting fetal vascular thrombosis (FVT) for chorionic plate/stem villous ectasia and thrombosis, and in formulating an overall

impression of FVT as the cause of stillbirth. 4 of 5 kappa values were substantially significant (.6-.8) to nearly perfect (.8-1.0) in assessing interobserver variability of characteristics of FVT in stillbirth. The senior author's kappa value for intraobserver variability for each characteristic (data not shown) was .8-.92 for each category. Previously defined histologic placenta criteria for defining stillbirths due to FVT perform with statistical confidence and reproducibility.

13 Aberrant Expression of MUC1 Mucin in Pediatric Inflammatory Bowel Disease

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Background: MUC1 glycoprotein is normally expressed at low levels on the luminal side of healthy colonic epithelial cells. In colon cancer and other epithelial tumors, MUC1 is overexpressed and hypoglycosylated. We hypothesized that MUC1 expression might be altered in chronic inflammation, such as inflammatory bowel disease (IBD) and might contribute to the increased risk for colitis associated colon cancer.

Design: Colonic biopsies from pediatric patients with a clinical diagnosis of IBD (ulcerative colitis (UC), Crohn's disease (CD) and indeterminate colitis (ID)) as well as children with normal colonic biopsies and other colonic inflammatory conditions such as lymphocytic colitis and microscopic colitis were histologically studied to determine the diagnosis. The cases were stained by immunohistochemistry for MUC1 expression using the anti-MUC1 antibodies HMPV and 3C6 that recognize epitopes expressed on all forms of MUC1, and antibody 4H5 which recognizes an epitope expressed primarily on the abnormal hypoglycosylated MUC1. A positive and negative control was run with each testing.

Results: Normal colon showed low level apical staining in 3/5 samples, only with antibodies recognizing the normal form of MUC1. There was no staining in any of the normal samples for the abnormal hypoglycosylated form of MUC1. In IBD, normal MUC1 expression (3C6 and HMPV antibodies) was seen in 6/14 UC biopsies, 8/15 CD and 4/5 ID cases. The apical pattern was lost. Antibody specific for the hypoglycosylated form stained 9/14 UC, 7/15 CD and 3/5 ID cases. Non-IBD cases such as lymphocytic colitis showed staining for the normal MUC1 in 4/7 cases and only 1/7 cases showed weak focal staining for the abnormal form.

Conclusions: Pediatric IBD, like adult IBD, is characterized by overexpression of an abnormal, hypoglycosylated form of MUC1, previously documented to play an important role in colon carcinogenesis. This suggests a potential role of this molecule in the pathogenesis of IBD that has not been investigated to date.

14 Lymphocytic Gastritis In Pediatric Celiac Disease

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Background: An increase in gastric intraepithelial lymphocytes has been observed in some patients with the typical small

intestinal changes of celiac disease. To date, no clinical parameters have been described that identify the subset of patients more likely to have gastric involvement. The purpose of this study is to describe the clinical features of patients with celiac disease and lymphocytic gastritis, and to determine if the presence of lymphocytic gastritis portends a more severe form of celiac disease.

Design: We reviewed the pathology reports of all 192 patients with biopsy-proven celiac disease diagnosed in the last seven years at our institution. All patients received full upper gastrointestinal endoscopic study. Thirty-nine of these patients had lymphocytic gastritis. They were compared to patients with celiac disease without gastric involvement using the following clinical parameters, when available: age at diagnosis, initial anti-tissue transglutaminase (TTG) IgA levels, anti-endomysial IgA (EMA) levels, albumin, alanine transferase (ALT), aspartate aminotransferase (AST), hemoglobin, mean corpuscular volume (MCV), and symptom duration.

Results: Celiac disease patients with lymphocytic gastritis were more likely to be diagnosed at an earlier age compared to those without gastric involvement (5.3 +/- 0.9 years, n=22 versus 10.5 +/- 0.4 years, n=147; p<0.0001), have higher anti-TTG levels (591 +/- 68 units, n=22 versus 293 +/- 23 units, n=147; p<0.0001), higher anti-EMA levels (589 +/- 37 units, n=18 versus 308 +/- 23 units, n=133; p<0.0001), higher ALT (53 +/- 14 U/L, n=10 versus 25 +/- 2 U/L, n=52), higher AST (64 +/- 14 U/L, n=10 versus 36 +/- 2 U/L, n=52; p<0.001) and lower albumin (3.4 +/- 0.3 g/dL, n=10 versus 4.2 +/- 0.1 g/dL, n=52; p<0.0001) and MCV (79 +/- 2 fL, n=9 versus 82 +/- 1 fL, n=63; p<0.001) at presentation.

There were no statistically significant differences between the groups with respect to hemoglobin levels at presentation or reported symptom duration.

Conclusion: In the pediatric population, lymphocytic gastritis is associated with a more severe form of celiac disease, including an earlier age at onset and more profound laboratory findings compared to celiac disease without gastric involvement.

15 Liver Pathology in Infantile Mitochondrial DNA Depletion Syndrome

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Background: Mitochondrial DNA (mtDNA) depletion syndrome is a rare and relatively novel cause of hepatic dysfunction in the pediatric population. It comprises a heterogeneous group of disorders that are unified by a quantitative reduction in mitochondrial DNA and, thereby, dysfunctional respiratory chain. These disorders may be inherited in an autosomal recessive fashion or develop due to sporadic mutations. Patients typically present in the neonatal and early childhood periods with lethargy, jaundice, and often encephalomyopathy. The rapid decline in clinical course requires early recognition of its clinical, histologic, and ultrastructural features.

Design: Three children with mtDNA depletion syndrome treated at our institution from 2002 to 2006 were identified and the histologic, ultrastructural, and clinical findings were reviewed.

Results: All three patients presented in the immediate postnatal period with hypoglycemia and lactic acidosis. Histologic features of antemortem liver biopsies and postmortem autopsy specimens revealed steatosis, varying degrees of cytoplasmic oncocyctic change within hepatocytes, and minimal to mild inflammation. The presence of cholestasis and fibrosis were variable and the third patient also demonstrated bile duct paucity. Ultrastructural examination of livers from patients 2 and 3 further showed a marked increase in the number of mitochondria and decreased mitochondrial cristae.

Table 1. Clinicopathologic Features of mtDNA Depletion Syndrome

Age at Presentation	Lactate	LFTs	Fibrosis	Increased Mitochondria	Mutant Gene	Age at Death
Pt 1* DOL 1	Increased	Normal	None	NP	POLG	2 mos
Pt 2 DOL 5	Increased	Increased	+++	+++	MPV17	9 mos
Pt 3^ DOL 1	Increased	Increased	++	+++	dGK	1 year

* Liver only examined at the time of autopsy, diagnosis based on muscle biopsy.

^ Patient lost to follow up. Autopsy not performed.

NP B Not performed; LFTs B Liver Function Tests

Conclusion: Mitochondrial DNA depletion syndromes in the pediatric population are a heterogeneous group of disorders. Their unifying features include rapid clinical decline, steatosis, oncocyctic hepatocyte change, and marked increase in mitochondrial number with decreased cristae. All three of our patients expired by 1 year of life. Recognizing these features will aid in establishing prompt diagnoses and appropriate therapy.

16 Histopathological Alterations of Intrahepatic Biliary Tree in Heterotaxy Syndrome

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Background: Heterotaxy syndrome results from failure to establish normal right-left asymmetry during embryological development. Typical features include malposition or ambiguous situs of abdominal and thoracic organs, including midline symmetric liver, intestinal malrotation, and almost always asplenia or polysplenia. Biliary atresia has been associated with heterotaxy syndrome, but detailed descriptions of bile duct abnormalities in heterotaxy syndrome are lacking.

Design: The hospital autopsy database between 3/87-6/07 was searched for "heterotaxy". Demographic data was obtained from autopsy reports, and relevant phenotypic characteristics such as number of spleens, integrity of the extrahepatic biliary tree, liver and portal vein anatomy were recorded. Liver histology was evaluated by three pathologists, and the intrahepatic biliary tree was assessed by consensus. The following categories were used: normal, paucity of interlobular bile ducts (interlobular bile ducts in $\leq 50\%$ of portal tracts), and bile ductular proliferation with or without evidence of biliary dysgenesis. Control livers were also examined for comparison. Descriptive statistical analyses were performed.

Results: 55 autopsies from patients with heterotaxy were identified, but 15 were excluded because autopsy restrictions prohibited sampling of the liver. Of the remaining 40 patients (23 with polysplenia, 14 with asplenia, 3 with a single spleen), 16 (40%) had intrahepatic biliary tract abnormalities of which only 3 (7.5%) also had extrahepatic biliary atresia. Among the 16 patients with biliary abnormalities, paucity of interlobular

bile ducts was seen in 6/16 (38%), and occurred only with polysplenia. Bile ductular proliferation was seen in the remaining 10 patients, in 7 of which bile ductular profiles were markedly irregular and abnormal. These dysgenetic features were seen in all three patients with extrahepatic biliary atresia, and additionally in one patient with cystic dysplasia of the kidneys and pancreas (Ivemark II). Overall, intrahepatic bile duct abnormalities were seen more frequently with polysplenia (N=13) than asplenia (N=3).

Conclusion: Abnormalities of the intrahepatic biliary tree are common in heterotaxy syndrome (40%), even in the absence of extrahepatic biliary atresia, and are most strongly associated with polysplenia. The pathogenesis of such biliary dysgenesis is unclear, but may be associated with genetic and/or perfusion abnormalities in the liver.

17 Comparison of Calretinin Immunostain to ACE Histochemistry in Hirschsprung Disease and Controls.

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Background: The diagnosis of Hirschsprung Disease (HD) in suction rectal biopsies may be challenging. Acetylcholinesterase histochemistry (ACE) is a useful tool in this context but requires technical skill and observer experience. Calretinin (a calcium binding protein) immunohistochemistry, recently has been examined in distal rectal biopsies from infants with HD. Barshack (*J Clin Path* 2004;57:712-16) observed lost expression in nerves in the muscularis propria and submucosa of aganglionic segments. Luquette (*Ped Dev Path* 2007;10(1):76-77) extended this observation to include loss of expression in lamina propria nerve fibers.

Design: We examined 61 suction rectal biopsies (23 short segment HD, 6 HD in Down syndrome, 6 total colonic HD, and 36 ganglionated controls) using a pre-diluted Calretinin antibody (Cell Marque). The sections were scored blindly for Calretinin in ganglion cells and nerve stroma in the submucosa, and nerve fibers in the lamina propria. Findings were compared to the original H&E and ACE interpretations for concordance.

Results: Calretinin positivity of submucosal ganglion cells in the control group was 87% (27/31) with 65% (20/31) showing concomitant nerve stroma positivity. Interestingly, 68% (23/34) of control cases also showed calretinin expression in fibers within the lamina propria. In short segment HD we observed loss of calretinin expression of the submucosal nerve stroma in 83% (19/23). Also, loss of expression in lamina propria fibers was present in 96% of cases. In all 6 cases of total colonic HD no calretinin expression was present in the submucosal nerve fibers or lamina propria nerve twigs. However, in the Down syndrome group with HD, 50% (3/6) showed no expression in the submucosal ganglion or nerve stroma, and 67% (4/6) showed loss in lamina propria nerve fibers. In total, 32 of 35 cases of HD or 91% showed loss of expression of lamina propria nerve fibers. In comparison, the ACE histochemistry in all cases of HD was considered diagnostic of HD and normal in all controls.

Conclusion: Extending previously published work in a larger series of cases, we show that the loss of expression of calretinin in both submucosal ganglion/nerve stroma (87%) and lamina propria fibers (91%) is a very helpful but not an absolute sign

of HD when compared to the parallel evaluation of the ACE reaction in frozen sections. The loss of calretinin reactivity was less dramatic in HD associated with Down syndrome.

18 Wnt/ β -catenin Aberrations in Pediatric Liver Diseases

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Background: Wnt/ β -catenin signaling has come in the limelight for its role in normal liver development and regeneration where it dictates key processes of proliferation, apoptosis, zonation and metabolism. The aim of our present study was to address Wnt/ β -catenin signaling in a broad group of pediatric hepatic diseases ranging from metabolic disorders to malignancies.

Design: We utilize immunohistochemistry (IHC) for β -catenin and its surrogate target Glutamine synthase (GS), to evaluate status of canonical pathway in pediatric hepatocellular cancer (HCC) (n=9), hepatoblastoma (HB) (n=10), tyrosinemia (n=6) and miscellaneous conditions including progressive familial intrahepatic cholestasis (PFIC), glycogen storage disease (GSD), cystic fibrosis (CF), α 1-antitrypsin deficiency (A1AT) and hepatic fibrosis due to adrenoleukodystrophy.

Results: Normal donor livers displayed β -catenin at hepatocyte membrane and in cytoplasm in the biliary epithelial cells. GS was localized in a single layer of pericentral hepatocytes in normal livers. Two of the 9 HCC showed cytoplasmic/nuclear localization of β -catenin, which coincided with strong, diffuse GS localization. The HCC in these patients was in the background of TPN and tyrosinemia. All 10 HB showed strong nuclear and cytoplasmic β -catenin, however only 7/10 showed concomitant diffuse GS staining. Five of the 6 livers with tyrosinemia, some with dysplasia and one with HCC, showed cytoplasmic β -catenin, which correlated well with diffuse GS staining. For other metabolic disorders analyzed, both GSD livers and one PFIC liver showed diffuse GS staining with normal membranous β -catenin while livers from, A1AT, CF and adrenoleukodystrophy, showed no changes in β -catenin and GS.

Conclusion: We conclude that β -catenin activation may play a role in both neoplastic and non-neoplastic pediatric liver diseases and is highlighted by increased GS staining. We also believe that IHC for GS might be a better indicator of β -catenin activation in HB since nuclear β -catenin itself might be nonfunctional. Also, GS correlates well with β -catenin activation in HCC. β -catenin activation might be playing an important role in hepatic disease process in tyrosinemia and additional studies would be essential to identify the mechanism. Lastly, additional patients would need to be analyzed to define the mechanism of β -catenin independent increase in GS expression in GSD and PFIC as well as to verify any role in disease pathogenesis.

19 Histological Spectrum of Inflammatory Changes in Esophageal Crohn's Disease

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Background: Although the incidence of esophageal involvement in pediatric Crohn's Disease (CD) has been

reported to be as high as 40%, the histological changes, other than the identification of granulomas, are not well described. Our aim was to perform an in-depth histological study characterizing the spectrum of esophageal inflammatory changes in pediatric patients with untreated CD.

Design: The clinical charts and pathology reports of all children over 1-year of age, diagnosed with CD between 1/98-12/04, were reviewed. Only those children who had upper and lower gastrointestinal biopsies prior to any treatment were selected. Esophageal biopsies were blindly reviewed by 2 pathologists and categorized as either normal, i.e. defined as intraepithelial lymphocytes (IEL) $<6/HPF$ and no intraepithelial eosinophils (IEE), or abnormal. The abnormal biopsies were evaluated for the number of IEL and IEE, presence of intraepithelial neutrophils (IEN), eosinophilic superficial microabscesses, extent of inflammation, degree of basal layer hyperplasia (BLH), and presence of granulomas or multinucleated giant cells (MNGC) in the lamina propria.

Results: 166 children between 1 B 20 years of age (mean, 12 years) were identified. The esophageal biopsies were within normal limits in 62 (37 %) and were abnormal in 104 (63 %) cases. The lamina propria was included in 32 (19%) biopsies. Abnormal findings are listed in the table:

Histological Findings	Number of Cases	Percentage
IEL (6-20/HPF)	55/104	53 %
IEL ($>20/HPF$)	48/104	46 %
IEN	7/104	6.7 %
IEE (1-18/HPF)	18/104	17 %
BLH	34/104	32 %
Focal inflammation	65/104	62 %
Diffuse inflammation	39/104	38 %
Granulomas	3/32	9 %
MNGC	2/32	6 %

Conclusion: Esophageal involvement in pediatric CD is common. Compared to previous reports, granulomas were rare in this study. Large numbers of IEE, as seen in eosinophilic esophagitis, are not present in CD. Increased IEL constitute the predominant inflammatory change in esophageal biopsies from pediatric CD, and except for one, were observed in all abnormal biopsies in this study.

20 Necrotizing Enterocolitis in Premature Infants is Associated with a Relative Lack of FOXP3+ Regulatory T Cells in Surgically Resected Intestinal Tissue

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Background: Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency in the neonatal period and the exact pathogenesis is currently unknown. Immaturity of gastrointestinal immune regulation with an exaggerated host response to bacterial colonization and/or protein antigens may predispose preterm infants to NEC. FOXP3+ regulatory T cells (Treg) suppress innate and adaptive immune responses and are critical for intestinal immune homeostasis. The objective of this study was to test the hypothesis that premature infants with NEC lack Treg relative to other helper T cells and effector T cells in surgically resected intestinal tissue.

Design: In a retrospective case-control study, we performed immunohistochemistry on surgically resected intestinal tissue to blindly compare Treg to effector and helper T cell ratios in tissue from 18 infants with NEC and 32 control patients (infants with other surgical gastrointestinal problems such as bowel atresia or spontaneous intestinal perforation (SIP)). These specimens included biopsies and resections, obtained from both the large and small intestine, that were stained with antibodies to FOXP3 (1:700, eBioscience), CD4 (1:40, DakoCytomation), and CD8 (1:200, DakoCytomation) using tonsillar follicular tissue as the positive control. Data was compared between NEC and control groups with and without segregating by anatomic location.

Results: The corrected gestational age (CGA) of these patients ranged from 24.9 to 44.3 weeks, with the median CGA of 34.7 weeks. The median CGA of the NEC group was 31.3 weeks, and the median CGA of the control group was 36.0 weeks. Despite the lower CGA in the NEC group, the median number of Treg in NEC and control tissue was similar ($p=0.08$), indicating intact ontogeny of Treg in intestinal tissue early in gestation. In contrast, the total number of CD4+ T-helper cells was higher in the control group ($p < 0.001$), but the ratio (Treg/CD4) was not significantly different ($p=0.80$). Similar results were found with CD8+ T cells, with the total number of CD8+ effector T cells also higher in the control group ($p=0.0013$).

Conclusion: In contrast to mice, humans exhibit presence of intestinal Treg early in gestation. However, the lack of increase in the Treg/CD4 ratio in the lamina propria of NEC patients may indicate an insufficient Treg response to the hyper-inflammatory state associated with NEC.

21 RET Expression In Neuroblastic Tumors Correlates With Neuronal Differentiation And Maturation

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Background: RET encodes a tyrosine kinase receptor that is expressed in central and peripheral nervous systems neurons. In human neuroblastoma cell lines, differentiation appears to be coupled with increased RET expression. We stained human neuroblastic tumors using an antibody to RET protein product to determine if staining correlates with differentiation.

Design: The pathology database was searched for recent (1995-2006) pre-therapy neuroblastomas (NB), ganglioneuroblastomas (GNB) and ganglioneuromas (GN). Slides were reviewed to confirm the diagnosis and slides of representative blocks were stained with ret antibody. Fischer's exact test was used to determine differences between groups and significance set at $P<0.05$.

Results: 62 NB, 12 GNB and 8 GN were identified. MYCN status was available for 77 tumors, and 16 had MYCN amplified; 1/14 (7%) amplified cases with available blocks stained with ret, compared to 17/55 (31%) tumors with available blocks that did not exhibit MYCN amplification ($P>0.05$). Using the Shimada classification, 25 NB had favorable histology (FH), 30 unfavorable histology (UH) and 7 were unclassified. None of NB stained with ret antibody. In contrast, ganglion cells stained in all GNB ($n=11$) and GN ($n=7$). Among GNB, Schwannian stroma in 4/7 (57%) with FH

and 0/4 with UH stained with ret antibody ($P>0.05$).

Conclusion: RET protein expression correlates with neuronal maturation and differentiation in neuroblastic tumors. None of 62 NB stained with the antibody, regardless of histology or MYCN amplification. In contrast, all GNB and GN stained. Among all tumors, there was a trend toward ret staining in MYCN non-amplified tumors, and among GNB there was a trend toward stromal staining in FH tumors; the trends did not reach statistical significance in this study but significance might be achieved in a larger series. RET is a member of the tyrosine kinase category of cell receptors, and induction of RET expression may induce maturation in neuroblastic tumors.

22 GLUT-1 Immunoreactivity in Respiratory Hemangiomas and Hemangioma-like Lesions in Children

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Background: The differential diagnosis between juvenile capillary hemangioma (JCH), lobular capillary hemangioma/pyogenic granuloma (PG), congenital capillary hemangioma, and capillary vascular malformation can be difficult. In 2000, North et al. reported the expression of erythrocyte-type glucose transporter protein (GLUT-1) in JCH. However, these studies examined vascular lesions exclusively in skin and subcutaneous tissue. Since then GLUT-1 has been reported to be helpful to subclassify vascular lesions in liver, but to our knowledge no previous study has evaluated GLUT-1 immunoreactivity in respiratory tract hemangiomas. We propose to evaluate GLUT-1 expression in hemangiomas and hemangioma-like lesions of the respiratory tract in children.

Design: The computerized database in the Division of Pathology was searched for the diagnosis of hemangioma@ in all sites of the respiratory tract between 1983 and 2007. GLUT-1 stain was performed on all cases for this study. We reviewed the archived H&E stained slides and GLUT-1 staining patterns in each case.

Results: Forty-seven cases (23M/24F, mean age 3.2 y, range 2 wk-19 y) were studied. There were 20 cases each from nasal passage and larynx, 5 from oral cavity and 1 each from lung and bronchus. Strong linear endothelial staining for GLUT-1 was seen in 38 cases (nasal passage, 15; larynx, 20; oral cavity, 2; lung, 1). All were originally diagnosed as JCH except one from larynx, which was diagnosed as PG. Review with GLUT-1 immunostain confirmed that all 38 cases were JCH. Negative endothelial staining for GLUT-1 was seen in 9 cases: Two were originally diagnosed and later confirmed as PG (one each from bronchus and nasal passage). The other 7 were originally diagnosed as JCH. Review indicated that 4 of the 7 cases were PG (all from nasal passage); 2 cases were classified as capillary vascular malformation (both from oral cavity); one case from the oral cavity was reclassified as granulation tissue. In summary, GLUT-1 stain revealed 7 false positive cases in 9 non-JCH (78%), and one false negative case in 38 JCH (3%).

Conclusion: Our study indicates that respiratory JCH are most commonly seen in the larynx and nasal passage. The vast majority of vessels in lesions with typical histological features of JCH stain positive for GLUT-1. We also conclude that GLUT-1 immunostain is helpful because other vascular lesions, especially PG, capillary vascular malformation, and rarely granulation tissue may morphologically mimic JCH.

23 Isolated Tumor Cells Are Routinely Detected by Immunohistochemistry in Histologically Negative Bone Marrow Biopsies from Neuroblastoma Patients

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Background: Neuroblastoma is the most common extracranial malignancy in childhood. Both aspirate smears and trephine biopsy sections are vital components in the evaluation of metastatic disease; each with their own advantages. One of the biggest advantages provided by tissue biopsies is the ability to perform immunohistochemical analysis on fixed tissue. In this study, we sought to identify the frequency in which isolated tumor cells (ITCs) are identified with immunohistochemical methods, in histologically negative bone marrow biopsies. We also sought to retrospectively correlate the presence of ITCs with long term outcome.

Design: A total of 325 marrow biopsies from 51 patients (29 male, 22 female) during 180 separate clinic visits were identified. Slides from all tissue biopsies were reviewed and the clinical outcome was determined. Immunohistochemical studies using synaptophysin and chromogranin were performed on all biopsies (primary neuroblastoma resections used as positive controls) and isolated immunoreactive tumor cells were identified in histologically negative cases. The frequency of immunoreactivity to histologically unequivocal metastatic neuroblastoma was determined.

Results: Of 105 evaluations (85 bilateral biopsies and 20 unilateral biopsies, 190 total biopsies) resulting in negative metastases by routine histology, isolated tumor cells were present 18.1% of the time. Of 58 evaluations with metastatic disease identified by histology; synaptophysin and chromogranin showed immunoreactivity 98.2% and 79.3% of the time, respectively. Follow-up data was available in 48 of 51 patients, ranging from 6 to 152 months (average 59.3 months). Overall survival, as estimated by the Kaplan-Meier method, was not significantly different between patients with and without ITCs ($p = 0.357$). Patients with ITCs more often developed bone marrow recurrences (31%) versus patients without a history of ITCs (9%) ($p = 0.075$).

Conclusion: Routine H&E evaluation of bone marrow biopsies for staging/surveillance in neuroblastoma patients fails to identify isolated tumor cells nearly one fifth of the time. Immunohistochemistry using synaptophysin and chromogranin can reliably identify these ITCs, though their presence does not predict a significantly different overall survival. ITCs do, however, appear to correlate with an increased risk of recurrence within the marrow. While this latter finding is not statistically significant, it may represent a subset of patients in whom complete remission is difficult to attain. Further study is necessary to evaluate the validity of predicting recurrent disease through the identification of ITCs.

24 AP2 β Immunohistochemistry in Rhabdomyosarcoma cases.

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Background: AP2 β is a member of AP2 family of transcription factors, involved in cell growth, differentiation and

programmed death. Recently, by gene expression data, it has been found that AP2 β is expressed in fusion positive alveolar rhabdomyosarcoma (ARMS). In the process of segregating the two morphologic types of rhabdomyosarcoma (RMS) by differential immunohistochemical stains, we found that a significant number of ARMS cases with PAX3/PAX7 rearrangement stained positive for AP2 β .

Design: Forty IRSG registered cases of RMS between the years 1990 and 1996 with known gene fusion status were retrospectively examined for AP2 β immunoreactivity. Archived unstained paraffin sections were stained with antibody directed towards AP2 β by antigen retrieval method. The nuclear staining was semiquantitatively scored by two of the investigators.

Results: There were 10 cases of Embryonal RMS (ERMS) and 30 cases of ARMS including 20 known fusion positive cases. The positive staining pattern was crisp nuclear, and in most cases stained >70% of the tumor nuclei. Strong nuclear positivity was also present in crushed tumor tissue. The negative staining patterns were either completely negative or cytoplasmic granular staining in tumor cells. 16 of the 20 fusion positive ARMS were AP2 β positive, 3 cases were negative and 1 case was lacking tumor in the section. 9 of the 10 fusion negative ARMS were AP2 β negative and one case was positive. All the ERMS cases were negative.

Conclusion: AP2 β may differentiate fusion positive ARMS cases and may help in morphologic sub typing in difficult RMS cases, since ~80% of ARMS cases are fusion positive and behave aggressively. Future studies will include correlation between AP2 β and break-apart probe studies for FKHR by FISH on paraffin tissue.

25 Utility of Nestin Immunohistochemistry in Differentiating Pediatric Renal Tumours

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Background: Nestin is an intermediate filament protein that was first identified in neuroepithelial stem cells. During embryogenesis, nestin is expressed in a number of cell types, including neural crest cells, neuronal and glial precursors, and developing myocytes. It is not surprising, therefore, that nestin immunoreactivity has been described in pediatric brain tumours, neuroblastomas and rhabdomyosarcomas. We have recently shown that nestin is expressed in podocytes and nephrogenic blastema in human kidney. With this in mind, we studied the expression of nestin in a series of pediatric tumours to determine the utility of nestin expression in differentiating tumours in the region of the kidney.

Design: The cases studied included: Wilms tumour (n=14), renal cell carcinoma (RCC) (n=19), renal clear cell sarcoma (n=8), neuroblastoma (n=11), renal malignant rhabdoid tumour (n=2), Ewing sarcoma/PNET (n=14 including 3 renal and 11 extrarenal) and desmoplastic small round cell tumour (DSRCT)(n=5). Nestin expression was assessed by immunohistochemistry. Cytoplasmic staining for nestin was scored on a scale of 0 to 3+ as follows: 0 for no staining, 1+ for <10% tumour cells positive, 2+ for 10-50% of cells positive, 3+ for >50% of cells positive.

Results: The percentage of cases showing nestin expression for each tumour type is presented in the following table.

	0	1+	2+	3+
Wilms tumour	0	7	7	86
Neuroblastoma	0	0	9	91
Rhabdoid tumour	0	0	0	100
DSRCT	0	0	60	40
Clear cell sarcoma	50	13	13	25
Ewing sarcoma/PNET	71	29	0	0
RCC	79	21	0	0

Staining of 2+ or 3+ intensity was seen in the majority of Wilms tumours and in all neuroblastomas, rhabdoid tumours and DSRCTs. In Wilms tumours, nestin staining was seen in blastema, stroma and primitive glomerular structures, but not in primitive tubules. In neuroblastoma, positive staining was detected, regardless of the degree of differentiation. All cases of RCC and Ewing sarcoma/PNET showed either 0 or 1+ staining. The staining pattern seen with clear cell sarcoma was extremely variable; half of these cases were nestin-negative whereas the other half showed 1-3+ staining.

Conclusion: Nestin appears to be a highly sensitive marker of Wilms tumour. However, its specificity for Wilms tumour is low, as it is expressed by a number of other tumours that may occur in or around the kidney. Positive staining for nestin may be useful in differentiating Wilms tumour from Ewing sarcoma, and negative staining may be useful in differentiating clear cell sarcoma from Wilms tumour. Nestin expression in malignant rhabdoid tumour and DCRST may reflect the neural and/or muscle phenotypic features that these tumours can express.

26 Immunohistochemical Analysis of 61 Clear Cell Sarcomas of the Kidney for a Panel Including NGFR and CD99

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Background: Clear cell sarcoma of the kidney (CCSK) is an uncommon, high-risk renal tumor of childhood. Recent studies based on a small number of cases suggest that CCSK may be neural in origin and that the nerve growth factor receptor (NGFR) may be a diagnostically useful marker. Other preliminary studies have demonstrated CD99 positivity in a small number of CCSKs. The present study comprehensively examines the utility of NGFR and CD99 in the diagnosis of CCSK, along with a panel of other immunohistochemical markers.

Design: A tissue microarray was created from 66 CCSKs, with all cases sampled in triplicate. Two consistently scored samples were required for each tumor to be considered evaluable for each marker. Controls included normal kidney, placenta, and a variety of pediatric tumors. Antibodies to NGFR, vimentin, CD99, BAF47, WT1, CD10, beta-catenin, Bcl-2, CD34, CD56 and nestin were analyzed. Staining was evaluated based on intensity, percentage of cells staining, and cellular localization.

Results: As expected, 61/66 CCSK study tumors were positive for vimentin; those negative for vimentin were excluded from further analysis. All CCSKs demonstrated nuclear positivity for BAF47. NGFR was diffusely positive in all CCSKs with 2-3+ staining intensity. No CCSK was positive for WT-1, beta-catenin, Bcl-2, CD34, or nestin. 57/60 CCSKs were negative for CD56. 54/59 CCSKs were negative for CD10. CD99 expression in CCSKs showed a continuum of staining,

with 12 negative tumors, 24 cases that showed 1+ staining, 17 cases that showed 2+ staining, and 5 cases that showed 3+ staining. Staining was seen in both cytoplasm and plasma membrane, although membranous staining was not as intense as typically is seen in cases of PNET.

Conclusions: NGFR represents the first reliably positive marker for CCSK. Additional studies evaluating other renal tumors are required to evaluate the specificity of NGFR. Useful negative markers include CD56, WT-1, Bcl2, and BAF47. CD99 staining may be positive in some CCSKs, although most show weak to no staining. These observations provide further evidence supporting the neural histogenesis of CCSK. The potential diagnostic utility of NGFR cannot be underestimated. CCSKs may show a variety of growth patterns, and are therefore often difficult to confidently identify. Reliable diagnosis of CCSK enables treatment with doxorubicin, which has had a positive impact on long-term survival.

27 Is MIB-1 Proliferation Index An Effective Substitute For Mitotic-Karyorrhectic Index (MKI) In Neuroblastoma Classification?

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Background: The International Neuroblastoma Pathology Classification (INPC) utilizes neuroblastic/stromal composition, differentiation, patient age, and MKI to classify neuroblastic tumors prognostically as having favorable or unfavorable histology. In principle, the MKI reflects proliferation and apoptosis. The process of counting 5000 cells is time-consuming and can be difficult in small or poorly preserved specimens. If proliferation index, an easily measured parameter, correlates well with MKI in abbreviated counts, it could provide a meaningful substitute for formal MKI determination in routine practice.

Design: We identified 27 neuroblastoma cases from departmental archives for which formalin-fixed, paraffin-embedded tissue was available. Two authors independently counted mitotic/karyorrhectic figures in separate 500, 1000, and 5000-cell manual counts at 400x on H&E-stained slides. Immunostaining using an antibody against MIB-1 (Ki-67), a proliferation marker, was performed on an automated Dako system using the manufacturer's recommended protocols. Positive nuclei were enumerated in 500 and 1000 cell manual counts. Correlations between arrays of counts were analyzed statistically via Pearson coefficients.

Results: Abbreviated MKI counts (500 or 1000 cells) correlated poorly with full 5000-cell counts ($r^2=0.37$ and 0.42 , respectively). Likewise, MIB-1 staining did not correlate well with the 5000-cell MKI counts ($r^2=0.25$ and 0.38). If MIB-1 staining of 1000 cells was categorized as low (<10% of nuclei), intermediate (10-60%), or high (>60%), and used in lieu of the corresponding MKI categories, there was 100% concordance between the results of the INPC using MKIs and a modified INPC using proliferation indices. Categorization by 500-cell MIB-1 counts was only slightly less accurate (95% agreement).

Conclusion: The MKI, while a simple histologic concept, can be difficult and time-consuming to determine. Proliferation index assessment, using MIB-1 immunohistochemistry, is faster, easier to interpret, and requires counting fewer cells.

Furthermore, this method is amenable to automated quantitation. These findings need to be validated in a larger cohort but in our study, MIB-1 proliferation index served as an effective substitute for MKI determination and allowed accurate classification of tumors into favorable or unfavorable histology.

28 Assessment of nuclear N-Myc protein concentration in neuroblastomas by quantitative immunofluorescence and comparison to MYCN copy number

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Background: Amplification of MYCN in neuroblastoma has a significant impact on patient prognosis, and is associated with increased risk of metastasis and rapid disease progression. Along with the International Neuroblastoma Pathology Classification, MYCN amplification status plays an important role in patient assessment and stratification. While gene amplification is logically assumed to correlate with concentrations of the protein product, in this case, the N-Myc protein, studies in other neoplasms demonstrate that there can be discordance. Comparison of N-Myc protein expression and MYCN copy number may provide additional insights into potential neuroblastoma behavior.

Design: Archival cases of formalin fixed, paraffin embedded (FFPE) tissue (n=64) were used to construct tissue microarrays (TMA). These cases represent a spectrum of neuroblastoma differentiation, including poorly differentiated and ganglioneuroblastoma cases. Amplification of MYCN was assessed using standard FISH and CISH assays. A polyclonal antibody was raised against a unique domain of the N-Myc protein. The antibody was validated for use in immunohistochemistry and the TMA was analyzed using objective quantitative analysis using immunofluorescence [AQUA(tm)]. The AQUA(tm) system provides a dynamic continuous measurement of subcellular staining within tumor cells identified by a second antibody (Amask@). The AQUA(tm) score is recorded as pixel fluorescence intensity (FI) within a subcellular location; in this study, nuclear FI.

Results: Our cohort of neuroblastomas demonstrated wide variation in MYCN amplification status, with approximately 22% demonstrating an increased number of gene copies. There was also wide variation in the AQUA(tm) score in these patients. The mean nuclear N-Myc protein concentration for neuroblastomas with MYCN amplification (804 +/-90.24 FI; n=17) was significantly higher than that in neuroblastomas with non-amplified MYCN (534 +/- 43 FI; n=47; p < 0.0089). However, a number of neuroblastomas demonstrated discrepancies between the N-Myc protein levels and MYCN amplification; i.e. patients with elevated N-Myc protein levels without evidence of gene amplification and vice versa.

Conclusion: Our results demonstrate a robust quantitative immunofluorescence method for measuring the nuclear N-Myc gene product in FFPE tissue. In general, N-Myc protein concentration correlates with MYCN amplification status, as expected. However, some neuroblastomas with non-amplified MYCN can show elevated N-Myc protein. Further work is required to determine the prognostic significance of these findings.

Poster Presentations

29 Association of High Expression of Bcl-6 and/or CD10 with Monomorphic Post Transplant Lymphoproliferative Disorders in Pediatric Patients

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Background: Bcl-6, a transcriptional repressor and CD10, a cell surface metalloprotease enzyme are known to play an important role in the regulation of germinal center (GC) B-cell differentiation, including antigen dependent proliferation. Bcl-6 and/or CD10 are frequently expressed in Burkitt's lymphomas, diffuse large B-cell lymphomas, and follicular center lymphomas. However, the expression of Bcl-6 and/or CD10 in post transplant lymphoproliferative disorders (PTLDs) has not been extensively studied. To investigate the frequency and significance of expression of Bcl-6 and/or CD10 in pediatric patients with PTLDs, we compared the expression of Bcl-6 and/or CD10 between morphologic subtypes of PTLDs.

Design: 39 cases of PTLDs (17 monomorphic B-cell cases and 22 polymorphic cases) diagnosed at Denver Children's Hospital were assessed for Bcl-6 and CD10 expression by immunohistochemical stains and/or flow cytometrical analysis. High expression (HE, 2:25% positive cells) of Bcl-6 and/or CD10 was compared between monomorphic and polymorphic groups.

Results:

	Monomorphic PTLDs	Polymorphic PTLDs	P value
Bcl-6 HE	8/17 (47%)	1/22 (4.5%)	0.0026
CD10 HE	9/17 (53%)	1/22 (4.5%)	0.0009

Conclusions: High expression of Bcl-6 and/or CD10 is more frequently seen in pediatric monomorphic B-cell PTLDs, suggesting that the germinal center cells appear to be more sensitive to EBV induced malignant transformation than other lymphocytes.

30 Transbronchial Biopsy in Infant Lung and Heart-Lung Transplant Recipients

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Background: The current experience with lung and heart-lung transplants is very limited in infants less than 1 year with only 10 reported in 2005 and 2006. Transbronchial biopsy procedures present unique challenges in these young infants and their contributions to patient management have not been well-studied.

Design: Sixteen transbronchial biopsies were studied from 4 patients (3 males, 1 female) who were transplanted (3 heart-lung, 1 lung) for pulmonary hypertension, congenital cardiovascular disease or surfactant protein abnormality (n=2) at a mean age of 6 months (range 3.5 to 9 months). Formalin-fixed, paraffin-embedded sections were stained with H&E (4 levels), trichrome and elastic stains, as well as stains for bacteria, fungi, and acid-fast bacteria. A fresh frozen piece was evaluated by immunofluorescence for immunoglobulin and complement deposition.

Results: The number of biopsy procedures ranged from 2 to 8 per patient. The mean age at the most recent biopsy was 18 months (range 11 to 22 months). At least 5 tissue pieces were

obtained in 81% and 3 pieces were obtained in 12% of the biopsies. Acute rejection was graded A0 (n=15) or AX (n=1, no alveolated parenchyma). Airway inflammation was graded B0 (n=8), B1 (n=2), or BX (n=6, no airway wall present). Open lung biopsy showed acute capillaritis with positive C4d in one patient and abundant foamy alveolar macrophages in a second patient. Additional studies documented gastroesophageal reflux in the second patient. In each patient, post-treatment transbronchial biopsies documented resolution of the pathologic process. A third patient had airway inflammation (B1) on 2 biopsies along with positive microbiologic cultures. Following treatment for infection and augmented immunosuppression, the airway inflammation resolved.

Conclusion: Transbronchial biopsies in 3 of the 4 patients documented efficacy of therapy and the large majority of biopsies were adequate to grade acute rejection. Transbronchial biopsies in infant lung and heart-lung transplant recipients provide critical information for clinical management.

31 Sclerosing Stromal Tumor: An important differential diagnosis of ovarian neoplasms Frequently Missed in Childhood and Adolescence

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Background: Sclerosing stromal tumors (SST) are rare benign stromal tumors that are clinically and pathologically distinct from fibromas and thecomas. More than 80% arise in young women with an average age of 27 years. Occasionally, they can occur in adolescence or premenarchal girls. Hormonal production is uncommon with only a few cases reported to produce estrogen and/or androgen or show elevated CA-125. Clinical symptoms include menstrual irregularities, abdominal discomfort and rarely ascites. Imaging studies frequently reveal solid or complex cystic adnexal masses with marked vascularity raising concern for germ cell tumors and, especially in the absence of elevated tumor markers, surface epithelial neoplasms. The differential diagnosis of a benign SST is seldom entertained.

Design: Clinical, imaging and pathological features were studied in four adolescent patients with SST initially presenting as unilateral adnexal masses.

Results: All four cases of SST were considered preoperatively potential malignancies. Histological analysis revealed the characteristic cellular pseudobulbar pattern composed of fibroblasts and round cells separated by densely collagenous or markedly edematous hypocellular tissue and prominent vascularity. The clinicopathological features are summarized in the table.

Case	Age	Gross findings	Clinical manifestations	Imaging	Other studies
1	13	7 cm tan-yellow	edematous	Menorrhagia x 4 month	Complex adnexal mass on MRI
2	17	15 cm tan-white cystic	Asymptomatic.	Found on routine GYN examination.	Complex cystic mass with thick enhancing irregular wall and septa on MRI
3	20	13 cm tan solid and cystic	Metrorrhagia,	abdominal fullness	Large solid pelvic mass and ascites on US
4	18	3.5 cm tan edematous	Pelvic pain	Adnexal mass on US	

Conclusion: Familiarity with SST is important when evaluating ovarian neoplasms in children and adolescents. Ultrasound, MRI and serum tumor markers are significant in the pre-operative evaluation; however, they may be insufficient in ruling out a malignant process. Intraoperative frozen section is prudent to prevent extensive, unnecessary surgery, and preserve fertility.

32 Lymphocyte Behavior in Eosinophilic Esophagitis

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Background: It has been demonstrated that experimental eosinophilic esophagitis (EE) in mice is associated with an increase in lymphocyte subpopulations (B+, CD4+, and CD8+ cells). By contrast, in humans lymphopenia and restricted T-cell repertoires are often associated with severe eosinophilic disease. In this study we aim to evaluate the number of intra-epithelial lymphocytes in subjects with varying degrees of esophageal eosinophilia.

Design: All esophageal biopsies from patients with the diagnosis of EE (n=80) and two groups of subjects without EE during 2005 at our institution were reviewed. The biopsies were divided into four categories: Group 1 (n=47) children with no esophageal eosinophils, Group 2 (n=46) children with esophagitis without diagnosis of EE (< 5 eosinophils/ high power field (hpf). Group 3 (n=26) children with mild to moderate EE (> 15- < 30 eosinophils/hpf) and Group 4 (n=54) children with severe EE (> 30 eosinophils/hpf).

Results: Among the four groups, the mean lymphocyte counts were:

Group 1 (children with no esophageal eosinophils) 6.2, range 1-26 lymphocytes; Group 2 (children with esophagitis) 17.6, range 1-68 lymphocytes; Group 3 (mild-moderate EE) 11.2, range 3-24 lymphocytes; and Group 4 (severe EE) 8.1, range 4-14 lymphocytes. In patients with EE there was a negative linear relationship between the number of eosinophils versus the number of lymphocytes (Slope = - 0.03, 95% Confidence Intervals (- 0.007 to - 0.006), P= 0.02). Statistical Analysis was done using Prism 4.0 (Graphpad Software).

Conclusion: There appears to be an inverse relationship between the degree of esophageal eosinophilia and the number of esophageal lymphocytes. Further investigation is needed to determine whether esophageal lymphocytes are down regulated in eosinophilic esophagitis.

33 Characterization of Pre-B Cell Acute Lymphoblastic Leukemia MicroRNA Libraries Using High-Throughput Pyrosequencing and Specimen Barcoding

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Background: MicroRNAs are a class of recently discovered non-coding RNA molecules that act to regulate other genes. They have been shown to influence many aspects of normal cell physiology, and to be dysregulated in pathologic states from cancer to cardiac disease. We are studying the roles of microRNAs in hematopoietic malignancies, particularly pediatric pre-B cell lymphoblastic leukemia.

Design: We have cloned libraries of small RNA molecules from pediatric pre-B ALL blasts representing major cytogenetic categories of this malignancy, and are characterizing these libraries using high-throughput pyrosequencing. We have designed DNA sequence barcodes incorporated into the linkers used for cloning the small RNA libraries, so that libraries from different samples can be pooled together, sequenced en masse, and later deconvoluted.

Results: Our pilot experiment has generated over 300,000 sequences from 18 pooled pediatric pre-B ALL small RNA libraries. We are analyzing these data to describe the profiles of microRNA expression for each cytogenetic subtype.

Conclusion: The methods used in this study are well-suited to thorough exploration of complex sequence pools such as cellular microRNAs, and are also applicable to the characterization of other complex DNA or RNA sequence libraries in a variety of research contexts.

34 Preliminary Analysis of the ETV6-NTRK3 Transcript Within the Cellular and Classic Components of Mixed Congenital Mesoblastic Nephroma

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Background: Approximately 20% of congenital mesoblastic nephromas (CMN) contain both classic and cellular components and are termed mixed CMN. The genetic and prognostic features of cellular and classic CMN are different. Cellular CMNs are characterized by the ETV6-NTRK3 fusion gene, and no consistent genetic abnormalities have been identified in classic CMN. The pathogenesis of mixed CMNs is largely unknown.

Design: Eight mixed CMNs were identified for which the classic and cellular components could be separately analyzed for the ETV6-NTRK3 fusion product. Punches were taken from appropriate regions of paraffin blocks, and RNA extracted. Primers and a hydrolysis probe were designed for real-time RT-PCR detection of a 152bp segment of the fusion transcript. As an internal control, primers and a hydrolysis probe corresponding to a 165bp segment of mRNA for beta-actin were utilized. Two cases were run in duplicate using separate blocks. Five pure cellular CMNs were analyzed as positive controls, and normal liver tissue was utilized as a negative control.

Results: Surprisingly, each of the cellular and classic areas from the eight mixed CMNs demonstrated an absence of the ETV6-NTRK3 fusion transcripts, while the internal control was strongly positive in each case. The proportion of the cellular subtype varied from 5%-90%. The five pure cellular CMNs all demonstrated the fusion product.

Conclusions: The pathogenesis of mixed CMNs has been a point of speculation and controversy. First, it is not clear that a similar phenomenon within infantile fibrosarcomas at non-renal sites exists. (Similarly, it is not clear that an extrarenal equivalent to the classic subtype of CMN exists). Second, multiple foci of cellular histology are commonly distributed throughout mixed CMN, making clonal evolution less likely and more difficult to understand. This study suggests that the cellular subtype does not necessarily correlate with the

translocation responsible for the ETV6-NTRK3 fusion transcript. This may have prognostic and therapeutic implications for patients with mixed CMNs. However, analysis of a larger number of cases is necessary.

35 Cystic Nephroma Shows No Deletions in WTX

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Background: WTX is a recently discovered tumor suppressor gene shown to be mutated in about one-third of Wilms tumors (WT). Inactivation of WTX, which is located on the X chromosome at Xq11.1, is a Asingle-hit@ event occurring on the single X chromosome in tumors from males and the active X chromosome in tumors from females. The majority of mutations are deletions detectable by fluorescence in-situ hybridization (FISH). Cystic nephroma (CN), which like cystic partially differentiated nephroblastoma is considered part of the WT spectrum, has not been previously examined for WTX mutations.

Design: Clinicopathologic features of CN diagnosed at our institution between 1994 and 2007 were reviewed. Three-color FISH was performed on paraffin sections from 5 CN using a probe for WTX and probes for the X chromosome centromere and a telomeric site as controls; at least 30 nuclei were scored in each assay.

Results: Patients (4 boys and 1 girl) ranged in age from 7 months to 16 years (mean 10 years), and presented incidentally (n=3) or with gross hematuria (n=1) or a palpable mass (n=1). Grossly, the lesions were multiloculated cysts ranging from 3.0 to 7.2 cm (mean 5.7 cm). Microscopically, cystic spaces were lined by cuboidal epithelial cells; septa contained variably cellular fibromyxoid tissue with no blastema. FISH showed no deletions in WTX.

Conclusion: The lack of WTX deletions in our series of CN suggests that WTX is not mutated in CN. Differences in the integrity of WTX may account for differences in pathobiology between CN and WT.

36 Features, Associations and Complications of Craniolacunia: An Autopsy Series

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Background: Craniolacunia, or luckenschadel, is an unusual form of skull pathology characterized by well circumscribed thin areas (lacunae) in the calvaria. The pathogenesis of craniolacunia is not understood and most reported cases have been associated with either Chiari malformation, neural tube defects or craniosynostosis.

Design: Six cases of craniolacunia in the last 30 years were identified in the autopsy files of our pediatric hospital. Case histories, photographs, and where available, radiographs and histologic sections of the cranial bones were reviewed.

Results: Age at autopsy ranged from a 40-week stillborn fetus to a five year old child. In each case, lacunae were multiple and involved the parietal bones (five cases) or frontal bones (one case), in a symmetric distribution. Two cases were associated with Chiari malformation, including one in an infant with

limb-body wall complex. One case was in an infant with trisomy 18, with mild ventricular dilatation and small brainstem and cerebellum. None of the patients had craniosynostosis, although two were dolichocephalic. Three patients had no other congenital malformations, including a two day old term infant born by vacuum-assisted delivery. Autopsy of this baby disclosed a parietal bone fracture through a lacuna near the site of vacuum cup placement, with extensive subcutaneous hemorrhage. Histologic sections of the calvaria in two of the cases with no associated abnormality were compared with calvaria of normal, age-matched control infants. Lacunae were characterized by focal, dramatic thinning to complete absence of the calvarial bone (lacunae mean thickness = 165 microns; adjacent bone mean thickness = 705 microns). The controls and calvaria adjacent to lacunae consisted of trabeculae of lamellar bone with osteoblastic rimming and a paucicellular marrow space. By contrast, lacunae contained relatively cellular fibrous tissue and small discrete islands of woven bone. Sections of rib and vertebral body in each case showed normal ossification.

Conclusion: Our six cases of craniolacunias constitute the largest reported pathologic series and differ from other published cases in the following respects:

1. In contrast with the overwhelming majority of published cases, three cases (50%) in our series were not associated with any other congenital malformation, including craniosynostosis or abnormalities of the central nervous system.
2. This represents the first report of craniolacunia in association with trisomy 18.
3. Skull fracture through a lacuna during delivery has not been described previously. Craniolacunias, which can be diagnosed prenatally, may represent a risk factor for obstetrical injury.

37 Chromogenic in Situ Hybridization (CISH) for Detection of Epidermal Growth Factor Receptor (EGFR) Copy Number to Determine the Neoplastic Nature of Ganglion Cells in Glioneuronal Neoplasms

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Background: Gangliogliomas are rare tumors of the central nervous system. Histologically, they are composed of intimately admixed glial and neuronal components, the pathological origin of which remains largely unknown. Previous studies of ganglioglioma mainly focused on histologic features, immunohistochemical analysis, clinical treatment, and patient outcome. Very few cytogenetic and molecular genetic studies have been reported on this neoplasm. Whether the ganglion cell component in ganglioglioma is neoplastic is not confirmed with certainty. In an attempt to determine the neoplastic nature of the neuronal component of ganglioglioma, we studied the expression of EGFR gene copy number by using the chromogenic in situ hybridization method in 5 cases of ganglioglioma of the central nervous system.

Design: Five cases of histologically confirmed central nervous system gangliogliomas were collected from the files of the Brigham and Women's Hospital and Children's Hospital, Boston. Using the chromogenic in situ hybridization method, we counted the number of EGFR gene copy number in the glial and neuronal components of the gangliogliomas. The EGFR Amplification Probe (Zymed Laboratories Inc.) is used for this

purpose.

Results: In 4 out of 5 gangliogliomas, numerous neurons showed increased EGFR gene copy number. The number of EGFR gene copy number varied from 4-7 gene copies per neuron (median: 5 gene copies per neurons). All cells in the glioma component showed 2 EGFR gene copies. In one case, both the glial and neuronal component showed 2 EGFR gene copies.

Conclusion: Using the chromogenic in situ hybridization method, we found that many neurons in gangliogliomas of the central nervous system showed amplification of the EGFR gene confirming their neoplastic nature. Finding a simple technique to characterize the neuronal component of gangliogliomas as neoplastic is particularly important in cases where the neuronal component is minimal and there is doubt as whether this component represent a neoplastic process (ganglioglioma) or entrapped neurons by the glial neoplasm (astrocytoma or oligodendroglioma). The distinction between ganglioglioma and pure glial neoplasm is paramount as the prognosis and the treatment differ radically. In addition, this technique is relatively simple to perform on single cases and its interpretation requires simple light microscope.

38 Eosinophilic Esophagitis in a Pediatric Population of the Tennessee and Kentucky Region

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Background: Eosinophilic esophagitis (EE) is an esophageal inflammatory condition with a predominance of intraepithelial eosinophils. EE is unresponsive to acid suppression therapy and has been documented to affect adults as well as children. The exact pathogenesis of EE is unknown, but appears to be linked to allergic stimuli. First diagnosed in 1978, EE is gaining greater recognition as an entity apart from reflux esophagitis. The incidence and prevalence of EE appears to be rising, but there is insufficient data as to the prevalence of EE in children. This study describes the prevalence of EE in children undergoing endoscopy at our institution between the years 2001-2006, inclusive.

Design: A retrospective review of all previously collected pediatric esophageal biopsies performed at Vanderbilt University Hospital and Monroe Carell Children's Hospital at Vanderbilt from 2001-2006 was performed. A total of 3863 biopsies were identified. Only first time biopsies of patients below 18 years of age were evaluated. The eosinophil count was obtained from the pathology report. If the number of eosinophils was not available, the slides were reviewed. Biopsies with more than 20 eosinophils/HPF were considered to have EE.

Results: The overall prevalence during the study period was 8.5%. The prevalence (%) by year was as follows: 2001 = 7.7; 2002 = 6.9; 2003 = 6; 2004 = 5.9; 2005 = 7.3; 2006 = 17.2%.

Conclusion: It has been proposed that the prevalence of EE is on the rise. Our study shows a prevalence of approximately 7% from 2001 to 2005, and an increase to 17% in 2006. We are conducting further studies to document the prevalence of EE before 2001, and to see if the increase in the last year persists.

39 Animal Models of Viral Upper Airway Infection

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Background: Respiratory Syncytial Virus (RSV) infection is common in infants and young children with the majority of patients experiencing their first infection in infancy. In ~60% of these infants, infection is relatively mild and is limited to the upper respiratory tract. Severe RSV disease is a result of lower airway infection, which is largely prevented by the presence of neutralizing serum antibody (maternal, endogenous or administered). Interestingly, effective immunity to RSV infection of the upper airway is never established, and reinfection occurs throughout life. While upper airway infection is not life-threatening, there is a considerable disease burden associated with frequent infections for those patients prone to chronic otitis media or to asthma. Unfortunately, and largely due to the lack of an appropriate animal model, protection of the upper airway is an understudied area.

Design: In this study we used plaque assay and immunohistochemistry studies to compare the relative susceptibility of mice and cotton rats (*Sigmodon hispidus*) to upper and lower respiratory tract infection by RSV.

Results: We found that, unlike the mouse model where only rare nasal epithelial cells are infected by RSV and lower infection is largely limited to type I pneumocytes, there is extensive infection of the nasal epithelium in the cotton rat. Moreover, fewer than 50% of infected animals developed lower respiratory tract infection as determined by plaque assay and immunohistochemistry. Equally interesting was the finding that, even in animals with no evidence of pulmonary infection, there was evidence of airway inflammation.

Conclusion: The pattern of RSV infection seen in cotton rats more closely resembles the natural history of this disease in human patients and will provide a useful model for studying the pathogenesis of viral upper respiratory tract infection.

40 Strong Immunohistochemical Expression Of X-Linked Inhibitor Of Apoptosis In Neuroblastoma, Wilms Tumor, And Pediatric Classical Hodgkin Lymphoma

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Background: Many cancer therapeutic agents act by promoting apoptosis, and therefore, molecular alterations in the apoptotic machinery can make cancer cells resistant to therapy. Understanding the molecular mechanisms of such resistance can lead to the identification of potential therapeutic targets. Inhibitors of apoptosis (IAP) family of proteins are endogenous caspase inhibitors characterized by baculovirus IAP repeat (BIR) domains. The X-linked Inhibitor of apoptosis (XIAP), a member of the IAP family and one of the most powerful inhibitors of apoptosis, mediates its effects by inhibiting downstream caspases 9 and 3. It may be a key cause of resistance to chemo-radiation therapy-induced apoptosis in some adult cancers, but its expression in pediatric tumors has not been systematically investigated.

Design: Immunohistochemical staining for XIAP (1:100, BD Biosciences) was performed on 24 neuroblastomas (1

undifferentiated, 14 poorly differentiated, 9 differentiating), 30 Wilms tumors (including 5 with diffuse anaplasia and 1 with focal anaplasia), and 53 pediatric classical Hodgkin lymphomas (cHL) (39 nodular sclerosis, 14 mixed cellularity). The results were recorded as: absent (no staining), weak (faint and focal staining) and strong (intensely positive in most cells). For pediatric cHL, staining in Hodgkin/Reed-Sternberg cells was recorded.

Results: The neoplastic cells in the majority of all three pediatric tumors (67% of neuroblastomas, 93% of Wilms tumors, and 70% of pediatric cHL) showed strong reactivity for XIAP. Seven neuroblastomas (6 poorly differentiated and 1 differentiating) showed weak reactivity for XIAP, while one differentiating neuroblastoma was negative for XIAP. Two cases of Wilms tumor, one with focal anaplasia, showed weak staining. Four cases of cHL were negative for XIAP, while 12 showed weak reactivity.

Conclusion: XIAP is strongly expressed in most cases of neuroblastoma, Wilms tumor, and pediatric cHL. This suggests that small molecule inhibitors of XIAP may potentially be useful as adjuncts to conventional chemo-radiotherapy in inducing apoptosis in therapy-resistant cases.

41 Alterations In Cell Cycle Checkpoints In Hodgkin/Reed-Sternberg Cells Of Pediatric Classical Hodgkin Lymphoma: A Tissue Microarray-Based Study

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Background: It is remarkable that the presumed germinal center-derived Hodgkin/Reed Sternberg (HRS) cells in classical Hodgkin lymphoma (cHL) lack B-cell receptor expression, have high proliferation index, and escape apoptosis. This suggests a disruption of cell cycle regulation in HRS cells. The aim of this study was to evaluate the immunohistochemical expression pattern of cell cycle regulators in a cohort of pediatric cHL.

Design: Immunohistochemical stains were performed on tissue microarrays composed of 54 cases of pediatric cHL from 51 patients (age range 4-19 with a mean age 12 years; 26 females, 25 males; 39 nodular sclerosis, 12 mixed cellularity subtypes). The sections were stained for the following proteins: cyclin D1, cyclin D2, cyclin E, p16, p21, p27, p53, retinoblastoma protein (Rb), and Ki-67 by standard immunoperoxidase techniques. The results in HRS cells were recorded as the average of duplicate staining: absent (no staining), weak (faint and focal staining), and strong (intensely positive in most cells).

Results: As shown in the table, the majority of HRS cells in all cases were in cell cycle, demonstrated by the strong Ki-67 expression. The cyclins D2 and E, but not D1, were expressed in most cases, whereas the cyclin-dependent kinase inhibitors, p16, p21, and p27, showed absent to weak expressions. The expression of p53 was similar to these inhibitors.

%	D1	D2	E	p16	p21	p27	Ki-67
Absent	89	2	6	58	34	78	0
Weak	11	66	37	37	64	22	14
Strong	0	32	58	6	2	0	86

Conclusion: Our study demonstrates that HRS cells, in pediatric cHL, are in cell cycle with a high proliferation index that is attributable, at least in part, to the increased expression

of cyclins and the downregulation of cyclin-dependent kinase inhibitors involved in G0-G1 and G1-S checkpoints. These alterations in the cell cycle regulatory pathways may provide potential novel therapeutic targets in pediatric cHL.

42 Macrophage activation in pediatric acute liver failure of indeterminate cause: An immunohistochemical study with clinical correlation

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Background: Acute liver failure (ALF) is a potentially fatal disease. The etiologies of ALF in children vary with age. Viral infections, metabolic disorders, drug toxicity are common causes. However in approximately 50% of pediatric ALF cases, the cause cannot be determined. Recent studies of soluble CD163 have showed that activated macrophages, particularly uncontrolled macrophage activation as a result of impaired NK/T cell function, are involved in ALF.

Design: We studied 16 surgical specimens of liver tissue with a clinical diagnosis of acute liver failure of indeterminate cause at Cincinnati Children's Hospital Medical Center between July 2003 and December 2006. All the original slides were reviewed; one or two representative blocks were selected for immunohistochemical studies using antibodies to CD 68 and CD163. Relevant clinical information and laboratory test results were obtained through chart review.

Result: Patients' ages ranged from a few days to 16 year old with 11 males and 5 females. All cases demonstrated significant increase in macrophages highlighted by both CD68 and CD163. CD68 and CD163 revealed similar staining pattern of macrophages, and CD163 is superior to CD 68 in term of demonstrating hemophagocytic activity. In 12 cases, macrophages were markedly increased in number with a diffuse distribution pattern. The macrophages appeared activated and large in size. Out of the 12 cases, 7 cases met the diagnostic criteria for hemophagocytic lymphohistiocytosis (HLH) including 5 cases with decrease in natural killer cell function or abnormal perforin expression confirmed by laboratory tests. In 4 other cases, increase of macrophages was mainly confined in portal and periportal region; the macrophages were slender and inactive in appearance. In further evaluation, autoimmune antibody was detected in 3 patients and the other patient had a strong family history of autoimmune disease; all 4 patients didn't fit a diagnosis of HLH clinically.

Conclusion: Macrophage activation plays a significant role in a subset of pediatric patients with ALF. A marked increase of CD163 positive activated macrophages with a diffuse distribution pattern in liver tissue from a child with ALF is a strong indication that HLH may be a potential underlying cause of the ALF.

43 Congenital Lobar Emphysema with Pulmonary Interstitial Glycogenosis: Variant of Emphysema or Localized Glycogenosis?

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Background: Congenital lobar emphysema (CLE) presents

within the first few months of life with increasing respiratory distress, and characteristic imaging reflecting overinflation. The index case was from a 4 week-old male. Pathologic examination of a lobectomy specimen revealed histologic and ultrastructural features of pulmonary interstitial glycogenosis (PIG) without significant overinflation of the alveoli and a slightly increased radial alveolar count. As PIG has been described as a diffuse interstitial process [Am J Resp Crit Care Med 165:1557-1565,2002], we retrieved all cases of CLE diagnosed during the past 7 years from the files at the Hospital for Sick Children to determine whether this histological feature was present in other cases.

Design: Six additional cases of CLE were identified. Patients ranged in age from 2 weeks to 3 years. The affected lobes included the left upper, left lower and right upper. The slides were reviewed and a panel of stains applied, including PAS, PAS-D, CD68, CD45, keratin AE1/AE3 and vimentin. Electron microscopy was carried out on 2 cases that had appropriately processed tissue. Radial alveolar counts (RAC) were performed and compared with values from the literature.

Results: Four of 7 cases, including the index case, were found to have focal histologic features of PIG, namely expansion of the pulmonary interstitium by a population of round to spindle cells with bland nuclei. These cells contained PAS positive cytoplasmic material that was sensitive to diastase, consistent with glycogen, and showed positive immunoreactivity for vimentin. Other immunostains ruled out an inflammatory etiology. EM confirmed the presence of cytoplasmic monoparticulate glycogen and features of primitive mesenchymal cells in both thick and thin areas of interalveolar septae. Mean RACs ranged from 7.7 (2 week-old) to 10 (3 year-old); values greater than expected for age.

Conclusion: We have identified cases of clinical CLE with focal areas of PIG, a heretofore undescribed histological feature. Classic cases of PIG have been described in infants presenting with diffuse interstitial lung disease. We suggest that the Apolyalveolar@ pattern of CLE described in the literature may be related to PIG; alternatively, the clinical spectrum of PIG may include localized disease presenting as CLE.

44 Expression of the Melatonin Receptor 1A and Tryptophan Hydroxylase in Placentas from Fetus with Intra-uterine Stress

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Background: Melatonin crosses the placenta and enters the fetal circulation. Moreover, experimental data suggest a possible influence of melatonin on placental function and fetal organ development in humans. To date, the expression and role of melatonin receptors in human placenta and fetal organs in perinatal autopsies remain to be elucidated.

Design: To address the correlation between placental melatonin expression in different causes of perinatal death (Congenital Anomalies and Perinatal Stress, Ascending Infection or Perinatal Hypoxia/Anoxia) we investigated the expression of two types of membrane/cytoplasmic melatonin receptors, tryptophan hydroxylase (TH), the enzymes required for the conversion of serotonin to melatonin and peripheral melatonin receptor 1A (MR-1A) in the human placenta from autopsied

fetuses matched by gestational age. The results were expressed in average percentage of the area with positive cells (PAPC) identified in the placental cross section of the umbilical cord insertion.

Results: The anti-TH antibody showed a cytoplasm staining pattern in cytotrophoblast, syncytiotrophoblast and in the inflammatory cells of the intervillous space, while MR-1A showed a membranous staining for the same structures and also for vascular endothelium. The PAPC for TH and MR-1A was significantly greater in cases of intrauterine stress. No significant difference occurred between the PAPC of TH expression between full term and premature gestations. In contrast, the PAPC for receptor 1A expression was significantly greater in full term gestations. A significant positive correlation occurred between gestational age and the percentage of PAPC for receptor 1A expression in the placenta.

Conclusion: This study showed the "in situ" localization of the melatonin synthesis and action, thus reinforcing the hypothesis of the paracrine and autocrine function of this cell. It was also showed that the performance of melatonin does not depend on the type of stimulus, since it was part of the placenta response to different types of intrauterine stress.

45 Exercise Training Reduces Blood Pressure and Improves Placental Neovascularization in Pregnant Spontaneously Hypertensive Rats.

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Background: The main purpose of the present study was to assess the effects of exercise training by swimming on arterial pressure levels, arterial pressure (APV) and heart rate variabilities (HRV) and repercussions in the placentas and fetuses of spontaneously hypertensive rats (SHR).

Design: Nine female 32-36 weeks-old SHRs were submitted or not to a swimming protocol during 09 weeks. In last 03 weeks of the training protocol, after mating, all animals became pregnant, resulting in two experimental groups: pregnant hypertensive sedentary (PHS, n=4) and pregnant hypertensive trained (PHT, n=5) rats. After finishing the training protocol, all animals, under anesthesia, had cannulas introduced into the femoral artery to directly record arterial pressure (AP) and heart rate (HR). After twenty-four hours, AP and HR were continuously recorded in baseline conditions during 30 minutes. At the end, animals were euthanized and the placentas and fetuses were sent for morphological analysis. The placentas were measured, weighted and a cross section of the placental umbilical cord insertion was fixed in 4% formalin and processed for immunohistochemical staining with anti von Willebrand antibody. The number of vessels was accessed by percentage of endothelial cells positive for the antibody per field. The histological section was complete analyzed and the results were expressed as percentage of vascular positive cells per case.

Results: The baseline mean arterial pressure (MAP) in trained female rats was significantly lower (107 7mmHg) when compared to sedentary female rats (133 2mmHg; p=0.013). Regarding to APV, low frequency (LF) band of PHT group

showed power spectral density values significantly lower than that observed in PHS (6.8 2mmHg versus 16.5 3mmHg; respectively, p<0.05), while values of power spectral density in high frequency band (7.6"0.5mmHg) was significantly higher as compared to sedentary group (4.6"0.4mmHg, p=0.006). It was not observed significant differences in relation to HRV. The pregnant females submitted to the exercise training presented a significant increase in the number of vessels in their placentas when compared to sedentary SHR (3.6 1.8% versus 3.5 1.6%; respectively, p=0,021). The PHT group showed values significantly larger (3.0 2.8cm) of fetal length than matched PHS group of animals (2.5 2.0cm, p=0,018).

Conclusion: All of these data indicate that the exercise training during pregnancy in SHRs rats causes a decrease in the cardiovascular sympathetic modulation, which could contribute to the reduction of arterial pressure. In addition, exercise training was also able to increase the number of vessels into the placentas of trained pregnant SHR, suggesting an increased placental neovascularization, which could improve the fetal viability.

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46 Transient lymphangiogenesis is a component of the inflammatory tissue response to extravasated mucin in mucoceles

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Background: The mechanism of lymphangiogenesis is poorly understood and controversy exists whether it is part of the inflammatory response to tissue injury. Mucocele of oral mucosa is considered to be a good human model for angiogenesis, and one study concluded that lymphangiogenesis was not involved in this process. Utilizing novel immunemarkers specific to lymphatics we aimed to study if lymphangiogenesis plays a role in tissue response in mucoceles.

Design: Ten extravasated mucoceles were selected. After review of H&E-stained slides they were grouped by widely accepted histologic criteria of wound healing; early, intermediate and late. To identify lymphatic vessels we used novel lymphatic-endothelium-specific-antibodies (VEGFR3, PROX1, D2-40). To asses the proportion of lymphatic channels of all lesional vessels we used the panendothelial immunomarker CD31. The presence, distribution and proportion of lymphatic channels were assessed and compared between the groups.

Results: Early phase lesions (n=3) were characterized by the presence of centrally located abundant inflammatory cells dominated by mucin-laden macrophages with scattered peripheral thin-walled large and small vessels. The intermediate phase lesions (5) showed much more numerous small and markedly ectatic and tortuous thin-walled vessels than the early phase. The vessels were present throughout the lesions; some lined mucin pools and young granulation tissue resembling a lymphatic malformation. The late phase (2) consisted of a young myofibroblast network with collagen deposition within which were frequent, mostly round microvessels. In early phase lesions the lymphatic markers were positive in about half the vessels with no lymphatic channels identified centrally. Most of the vessels in the intermediate phase were highlighted by all immunemarkers confirming that the vast majority of the

abnormally dilated vessels were lymphatic. In the late phase nearly all the microvessels stained with CD31, but only rare channels were positive with lymphatic markers.

Conclusion: Lymphangiogenesis and lymphatic vessel regression was observed during mucocele evolution. The abundant newly formed ectatic lymphatic vessels seen in the intermediate phase may play a role in the clearance of extravasated material (mucin, edema and lymph fluid) and in the initiation of the young-fibroblast rich granulation tissue formation. However, their role appears to be limited to the granulation tissue remodeling phase.

47 Effects of chemotherapy during pregnancy on the placenta

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Background: The risks of chemotherapy during pregnancy for mother and fetus have been well documented. With exception of methotrexate, an agent with known trophoblast toxicity, the effects of chemotherapy on the placenta have not been described.

Design: We performed a detailed clinicopathologic analysis of the placenta following chemotherapy, with emphasis on the trophoblast. Charts were reviewed for type and timing of chemotherapy, type of malignancy, and fetal and pregnancy outcome. Placentas were studied by standard histopathologic analysis and computer-assisted morphometry of the trophoblast. Morphometry controls were age-matched normal or small-for-age placentas.

Results: Pregnant women (n = 8, age 17-40 y) were treated with a wide range of chemotherapy agents for carcinoma of breast, ovary (2), cervix, salivary gland, lymphoma (2) or rhabdomyosarcoma. In seven cases chemotherapy was limited to second and/or third trimester; in one case (rhabdomyosarcoma), drugs were given throughout the pregnancy. Only the latter case was associated with congenital anomalies (cleft lip and palate, and tracheo-esophageal fistula). Two pregnancies were carried to term while six patients delivered prematurely at < 36 weeks. Three of eight placentas were small-for-gestational-age. The placentas showed a range of non-specific findings, including villous edema, villous hypermaturity, extravillous trophoblast proliferation, thrombi and infarcts. Morphology and morphometry of placental trophoblast (villous and extravillous) following chemotherapy during 2nd and 3rd trimesters were similar to controls. In contrast, the placenta exposed to first trimester chemotherapy showed striking changes of the extravillous trophoblast of the chorion laeve, including marked cytoplasmic vacuolization and nuclear enlargement (mean nuclear area 67 μm^2 ; normal controls: 39 μm^2 ; 2nd and 3rd trimester chemotherapy: 42 μm^2), associated with FISH-demonstrated polyploidy. Villous trophoblast and extravillous trophoblast in other sites were unremarkable.

Conclusion: Our findings suggest that chemotherapy during the first trimester induces polyploidy of the chorion laeve extravillous trophoblast, likely related to direct exposure of these rapidly proliferating cells to mitosis-modulating agents. Chemotherapy in the 2nd and 3rd trimesters was associated with non-specific findings, including underdevelopment (3/8

cases); without appropriate controls (untreated patients with similar malignancies) the specific role of chemotherapy in this group is difficult to assess.

48 Maternal Floor Infarction (MFI) and Biochemical Markers for Adverse Outcome.

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Background: Maternal floor infarction, or massive perivillous fibrinoid deposition, of the placenta is a rare disorder associated with fetal death, intrauterine growth restriction (IUGR), and a high-risk of recurrence. Low maternal pregnancy-associated plasma protein-A (PAPP-A) in the first trimester and high alpha-fetoprotein (AFP) in the second trimester are associated with adverse pregnancy outcome and risk of fetal aneuploidy. We sought to evaluate whether there was an association between these pregnancy screening tests and the occurrence of MFI.

Design: 28 cases of MFI were identified from the files of Weill-Cornell Medical Center between 2002 and 2007. Pathologic diagnoses were confirmed by rereview of the slides. Chart reviews were performed to obtain clinical information as well as obtain first and second trimester screening results.

Results: Maternal age varied from 24 to 48 years (avg 30), gravidity from 1 to 8 (avg 2.9), parity from 0 to 3 (avg 1.0). As expected, 75% of cases were associated with IUGR, with 35% of cases at or below the 1st percentile at birth. Birth weights varied from <1 to the 39th percentile. 13 patients underwent screening; 5 patients had screens for both PAPP-A and AFP; 3/9 patients who had first trimester screening had low PAPP-A levels (levels <10%); 5/9 patients who had second trimester screening had elevated AFP levels (levels >2.5 MoM). In patients with both screens, either PAPP-A or the AFP were abnormal. In 2 patients, both were abnormal. Interestingly, maternal serum hCG and estriol, 2 other common screening markers, were normal in all patients studied.

Conclusion: Elevated AFP may occur secondary to disruption of the maternal-fetal interface and thus this may be the mechanism for the increase seen in MFI. The association of PAPP-A to poor outcome and growth restriction has previously been shown but not its association with MFI. We have shown that abnormalities in these early biochemical abnormalities are seen in MFI. These findings also have important implications in patients with a history of MFI, as abnormal values may suggest a recurrence while normal values may decrease the suspicion of a recurrence.

49 Vascular Endothelial Growth Factor Receptor 3 (VEGFR3) and Prox-1 antibodies are superior to D2-40 in Identifying Malformed Lymphatic Vessels in Cystic Hygromas

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Background: Cystic hygromas consists purely of lymphatic vessels based on developmental evidence providing model for investigating malformed lymphangiogenesis and to test antibody sensitivity/specificity to lymphatic endothelial cells

(LECs). We previously reported that D2-40 (D) antibody (ab) identifies LECs of lymphatic malformations with high specificity but only moderate sensitivity. The lack of sensitivity leads to frustration in practice when a definite distinction between venous and lymphatic malformation needs to be made. Novel regulators of lymphatic channel development have recently been identified including VEGFR3 (V) and Prox1 (P), which play crucial roles in the initiation of lymphangiogenesis. The aim of this study was to assess the sensitivity and specificity of V and P abs to LECs and to compare with those of D in cystic hygromas.

Design: 17 cystic hygromas from the head, neck and axilla were selected and subsequent sections stained with D, V, P and CD31 (C) abs, respectively. We assessed the overall staining of vessels by each vascular marker at low power. We identified 5 D+ and 5 D- dilated vessels per case and compared staining with V and P. To assess V and P ab specificity to LECs we examined arterial endothelial staining.

Results: The low power impression was that in the majority of cases V (cytoplasmic staining) and P (nuclear staining) stained more large vessels than D (15/17). Small vessel staining was uniformly + with all vascular markers. Eight had no or minimal D staining of large vessels. In these cases all the selected D-vessels stained + with V and 7 were P+. Nine cases had variable D staining of large vessels; in these the selected D- channels were all P+ and 8 were V+. Two had occasional areas of dilated vascular channels negative with all lymphoid markers but + with C. Arterial staining was completely P-; occasional arterial wall blush was seen with V, and C was uniformly +.

Conclusion: Both VEGFR3 and Prox1 abs have superior sensitivity for malformed large lymphatic vessels of cystic hygromas when compared to D2-40 ab. VEGFR3 and Prox1 abs also have high specificity for lymphatic endothelial cells and we recommend their use in practice; especially in combination because their staining characteristic is complementary. The presence of lymphatic-marker-negative large channels suggests that cystic hygromas may not be purely lymphatic but that venous elements could also be part of the vascular malformation.

50 A Mole or not a Mole- that is the question

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Background: A common scenario in a community hospital is that of a pregnant patient who presents with first or second trimester vaginal bleed and undergoes a D&C procedure after spontaneous abortion is confirmed by sonography. Cytogenetics is usually not performed unless there is a history of recurrent pregnancy loss. A majority of partial molar and some complete molar pregnancy is not suspected clinically in its early stage. Therefore, an appropriate diagnosis or suspicion of molar pregnancy by the pathologist is crucial for appropriate patient management. The conventional method of using histologic criteria to determine whether hydropic villi observed in samples of products of conception (POC) represent hydropic abortus (HA), complete mole (CM) or partial mole (PM) has become problematic in

recent years. Both inter-observer and intra-observer variability have been reported. The classic features of hydatidiform moles are only occasionally seen since most pregnancy losses are evacuated early (on the average of less than 12 week gestation). In addition, chromosomal aberrations such as monosomy X and autosomal trisomies share similar morphologic features with hydatidiform moles in their early phase. This changing profile of hydatidiform moles has led to under-diagnosis and over-diagnosis in many cases. The combined use of fluorescence in-situ hybridization (FISH) technology and p57 immunostain has allowed the distinction of HA from CM and from PM. A prospective study is performed to determine whether morphologic detection of chromosomal abnormalities can be improved and refined using feedback from FISH results.

Design: All POC cases showing suspicious molar morphology were sent for FISH using paraffin sections. Centromere specific probes for chromosome X-Y-18 and 13-16-21 (Vysis, Inc., U.S.A.) are used. The cases with normal FISH results were re-evaluated morphologically and either reclassified as HA or remained unchanged. P57 immunostain (Neomarkers, Fremont, CA) was performed to rule out CM for those cases which remained unchanged.

Results: From July 2005 to August 2007, 290 consecutive cases with suspicious molar morphology were analyzed using FISH. Abnormal results were obtained in 132 cases which included 39% triploidy, 27% monosomy X and 24% autosomal trisomies. The percentage of cases with abnormal FISH progressively increased from 37% in 2005 to 53% in 2007.

Conclusions: Using the feedback from FISH result has enabled the recognition of the early and subtle morphologic features of chromosomal abnormalities, including those of molar pregnancy. The algorithm of using p57 and FISH in conjunction with morphology has ensured the diagnostic accuracy of molar pregnancy and ultimately led to better patient care.

51 Circumferential Curvilinear Arrays (CCA) of Intrahepatic Bile Ducts (ductal plate malformation-like) are More Common in Large Portal Areas of Explants with Congenital than Postnatal Biliary Atresia.

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Background: Desmet proposed that the intrahepatic bile ducts in biliary atresia (BA) occur in the congenital but not the postnatal form of BA, and resemble ductal plate malformation (DPM). We occasionally have observed ductular changes that superficially resemble the DPM (our term, CCA) in BA. We tested Desmet's hypothesis by comparing bile duct patterns in explanted livers in 5 cases of BA with splenic malformation (BASM, mean age at explant, 232 days) to duct patterns in 7 explanted livers from patients with no clinical or operative features of congenital BA (mean age at explant, 422 days).

Methods: Paraffin sections from 32 blocks representing major liver lobes of each case were stained with anti-cytokeratin antibodies AE1/AE3 and CK7 to highlight the bile ducts and the ductular reaction at the portal margins. We graded the duct and ductular reaction in portal areas of all sizes and counted the number of portal areas with large and small CCA. We recorded

the number of portal areas larger and smaller than 300 microns in diameter with each form of CCA. Large CCA were more or less linear duct formations parallel to the lobular margins that mimicked the orientation of the embryonic ductal plate. Small CCA were tiny complete or almost complete ringlets of ductules around a core of stroma.

Results: Large CCA were present in 46/146 portal areas >300 microns in diameter in BASM vs 24/223 in the more common postnatal BA, a statistically significant difference. Small CCA were only common in the presence of a complex, almost decorative, uniform filagree pattern of reactive ductular proliferation. The filagree pattern was prevalent in 3/5 explants

with BASM and 2/7 BA controls.

Conclusion: The increased frequency of CCA that resemble DPM in large portal areas associates with BASM but these arrays are not exclusive to congenital BA. CCA may represent a persistence of embryonic ductal plate anatomy in both forms of BA, or may be an acquired lesion that is more common in BASM. The infrequency of large portal areas in biopsy specimens is an obstacle to repeating these observations in specimens obtained at the time of a Kasai procedure.